# THE DEVELOPMENT OF INTRARUMINAL BOLUSES FOR CATTLE AND SHEEP

by
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## DECLARATION

I hereby declare that the work presented in this thesis is original and was conducted under supervision by the author.

I also certify that no part of this thesis has been submitted previously for the award of a degree to any University.

Donald C. Lawson

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## SUMMARY

This thesis is principally concerned with the construction and development of a sustained release bolus system supplying a range of trace elements to ruminant livestock. The system is a patented invention of the University of Glasgow.

An initial literature review describes the essential features of copper, selenium and cobalt inadequacies in ruminants together with assessments of current methods of corrective supplementation. The other components of the bolus are inorganic salts of manganese, zinc and iodine together with Vitamins A, $D_3$ and E.

Section 1 describes the technology of construction. A compressed mixture of common inorganic salts in cylindrical form (25 mm diameter, 40-100 mm length) is coated by dipping in a polyester resin leaving one flat end uncoated. Release of material into the reticulo-rumen is partly by dissolution and partly by mutual erosion of two boluses administered together. The initial prototype contained copper oxide, manganese sulphate, zinc oxide, zinc sulphate, sodium selenite, cobalt sulphate and potassium iodide with Vitamins A, $D_3$ and E.

The polymer coating breaks off progressively from the flat end as the bolus dissolves/erodes to leave a constant surface area and results in a constant release of material. A particular feature of the system is that there is no permanent residue in the rumen.

A standardised system of pressing and coating of boluses is described together with an in vivo system of evaluation of bolus release rates. This involved administration of pairs of boluses to adult cows fitted with a rumen fistula to allow frequent withdrawal for examination and assessment of weight loss.

Section 2 presents results of the examination of a range of factors which might affect the rate of release of nutrients from the bolus. These included the number of coats of resin and the initial length of the bolus. The compositional specification of zinc oxide (a major ingredient) was found to be of great importance and strict control was necessary to produce the required overall release rate. Changes in the inclusion of zinc sulphate could also markedly affect the dissolution/erosion characteristics. An increased number (1-3) of boluses simultaneously administered and the presence of metallic residues (end weights, tubes, cylinders) from other bolus systems was demonstrated to greatly increase the rate of release of material from the bolus.

The final construction of the bolus system was such that two cylinders (85 g each) gave an estimated release of nutrients (mg/day) amounting to 160 Cu, 0.7 Co, 0.8 Se, 74 Mn, 118 Zn, and 2.2 I with (i.u./day) Vitamins A 4859, $D_3$ 972 and E 9.7 over a period of about 240 days. Calculations demonstrated that these amounts would be adequate to supplement the expected dry matter intakes of each of the mineral elements in deficient herbage up to the levels accepted as appropriate dietary allowances for 150 to 500 kg cattle.

Section 3 describes a series of trials at different sites with grazing cattle judged by the local veterinary surgeons to require supplementation with one or more of the trace elements. The adequacy of supplementation was assessed by the measurement of blood parameters in comparison with untreated animals and/or animals given alternative supplementation as injections or alternative boluses. It was concluded that the bolus system provided adequate copper and selenium to cattle (130-500 kg liveweight) as judged by the responses in plasma copper and glutathione peroxidase activity and that these responses were as favourable as those found by injection of copper and/or selenate containing products. No sound conclusions regarding the effectiveness of the bolus system in supplying cobalt could be made due to the possible limitations of the assay method (and its interpretation) for Vitamin $B_{12}$. There were no indications of cobalt inadequacy in the cattle.

Initial reports of some regurgitation of the bolus in wider commercial use prompted the development of a basal weight system which increased the overall initial density from about 2.6 to 2.9 g/ccm. Thereafter no regurgitation was reported. The end weight eventually disintegrates within the reticulo rumen.

Section 4 of the thesis examined the possibility that the bolus construction with a modified matrix might be capable of providing a constant release of a variety of medicaments. Limited exploratory trials were conducted to examine the possible inclusion of materials such as levamisole hydrochloride, ivermectin, oxfendazole, laidlomycin propionate and Vitamin E. Distinct possibilities were found but much further work would be required to establish formulations giving appropriate and constant release of materials in the normally accepted therapeutic range.

Section 5 examined the possibilities for the development of a comparable, but smaller (19 mm diameter) bolus appropriate for use in sheep. Regurgitation was a major problem but was effectively eliminated by increasing the overall density to

3.0 g/ccm. The cost of manufacture would be such as to allow the use of only a single bolus. The absence of loss by mutual erosion between two boluses was found to lead to little further weight loss in prototypes after about 60 days. Nevertheless, analyses of faeces and of livers recovered at slaughter demonstrated the effectiveness of the copper contained in the bolus. These were such as to give concern about the possibility of potential copper toxicity.

In Section 6 an assessment was made of the effectiveness of a high density carbo-wax matrix as a carrier for avoparcin in an alternative bolus system. Comparisons were made by evaluation of the avoparcin concentrations in faeces with that resulting from a constant daily in-feed addition of avoparcin. Assessment of faecal output indirectly by estimation of the chromium content of faeces resulting from constant addition of the inert marker to the constant diet given to all cattle showed the experimental bolus construction to be irregular and erratic in relation to the direct inclusion in feed.

# LITERATURE REVIEW

## Introduction

There are fifteen trace elements known to be essential for the physiological development and health of animals. Of these, copper, selenium and cobalt are of major agricultural significance (Scottish Agricultural Colleges and Scottish Agricultural Research Institutes, 1982).

The results of blood surveys of grazing cattle have shown that deficiencies of these trace elements occur over large areas of the United Kingdom (Thompson & Todd, 1976; Anderson, Berrett & Patterson, 1979; Bloxham, Davis & Stephenson, 1979; Leech et al., 1982; Clegg, Hunt & Herbert, 1983; Bain, Spence & Jones, 1986). Geochemical and soil survey results have confirmed this situation. (SAC/SARI, 1982; Leech and Thornton, 1987).

Despite this apparent awareness of the problem of trace element deficiencies, there are still numerous reports of copper, selenium and cobalt deficiencies in cattle being diagnosed by the veterinary investigation service (Scottish Veterinary Investigation Service, 1990a, SVIS, 1990b, Veterinary Investigation Service, 1990).

This section reviews the effects of deficiences of copper, selenium and cobalt in cattle and the methods available for the supplementation of grazing animals with these trace elements.

# COPPER

## Function

The role of copper within the animal body is multifaceted with numerous copper containing enzymes and copper requiring processes being essential for the normal functioning of the body.

Copper is involved in the redox processes of tissue respiration, in osteogenesis, pigmentation and keratinization of hair, stability of hypophyseal hormones in blood and has an important role in blood synthesis. The involvement of copper in the above is explained by it being an integral component of many enzymes including superoxide dismutase, lysl oxidase, tyrosinase, caeruloplasmin, xanthine oxidase, galactose oxidase and uricase. Copper ions are also required as specific activators for some enzymes, for example sulphide oxidase.

## Deficiency symptoms and responsive disorders

Many of the clinical signs of copper deficiency are non specific and do not allow differential diagnosis (Suttle, 1983a). However the common clinical symptoms are:

### Anaemia

In the bovine, copper deficient anaemia is macrocytic and hypochromic. It is seen only in cases of prolonged deficiency where critically low plasma copper concentrations and caeruloplasmin activities are seen. The anaemia produced is due to the low levels of caeruloplasmin. This is the major copper containing enzyme in blood plasma and contains eight atoms of copper per mole of enzyme. Caeruloplasmin is involved in the iron saturation of the transport protein transferrin and thus in the absorption and transport of iron as haemoglobin.

### Bone disorders

Brittleness of bones and spontaneous fractures have been reported in copper deficient grazing ruminants. These skeletal abnormalities are often more noticeable after the onset of diarrhoea (Marston, 1952). Reduction in the activities of the copper enzymes amine and lysl oxidase are involved. These enzymes are involved in the formation of the cross linkages which give collagen and elastin their stability and strength. Copper deficiency results therefore in a decrease in cross linkage formation and in bone strength. The major effects of these changes are seen in adult animals.

**Diarrhoea**

This is not seen in all cases of copper deficiency but when it occurs is very severe. It is often seen in situations where a high intake of molybdenum rather than low copper intake is the causative agent (Ferguson, Lewis & Watson, 1943). Diarrhoea can also be observed where low copper intake is the sole cause of deficiency (Fell, Dinsdale & Mills, 1975; Mills, Dalgarno & Wenham, 1976).

The mechanism by which copper deficiency results in diarrhoea is unclear. Possible factors involved are malabsorption (Fell et al., 1975) or disturbances in gastrointestinal function due to a fall in the concentration of noradrenaline seen in copper deficient steers (Lawrence, Davies & Mills, 1982). The pathological changes observed in the pancreas of cattle of low copper status (Fell et al., 1985) may also be relevant.

**Depigmentation of hair**

Achromotrichia, especially periorbital, is a common clinical sign of copper deficiency and is shown at a relatively early stage of copper depletion. This effect is explained by the action of another copper containing enzyme (tyrosinase) which is involved in the conversion of tyrosine to the pigment melanin.

**Cardiovascular disorders**

The effects of copper deficiency on the enzymes amine and lysl oxidase and the repercussions of their lowered activities are also seen within the cardiovascular system. In Australia, cardiac lesions are seen in copper deficient animals in what is described as 'falling disease'. In this syndrome sudden death occurs usually preceded by exercise or excitement (Bennets, Beck & Harley, 1948). The occurrence of this disease is generally localised within north west Australia but a similar disease is also seen in Florida (Becker, Henderson & Leighty, 1965). The weakening of connective tissues due to copper deficiency also affects the circulatory system with rupture of major arteries occurring in non ruminants. Gross degenerative changes within the system have however been observed in cattle (Mills, et al., 1976).

**Reproductive failure**

A high incidence of infertility has been observed in the areas of Australia subject to 'falling disease' (Bennets et al., 1941) and further evidence from that continent has shown that the fertility of copper deficient dairy cows responds to copper supplementation (Hunter, 1977). Such positive effects have not always been shown elsewhere (Phillippo et al., 1982; Whitaker, 1982). More recently high molybdenum intakes (5 mg/kg DM) have been shown to delay puberty and reduce the conception rate in heifers (Phillippo et al., 1987), No such effects however were seen in animals of a similarly low copper status when this was induced by high dietary iron

intakes (800 mg Fe/kg DM). This difference may offer an explanation for the contrasting earlier reports.

**Interaction of other trace elements with copper**

Interaction can be defined as the alteration of the requirement of one nutrient by another nutrient (Klevay, 1980). The main interactions which affect the grazing ruminants requirement for copper are those with molybdenum, sulphur and iron.

High molybdenum intakes have been shown to reduce copper availability to ruminants (Ferguson et al., 1943). This antagonistic effect also requires the presence of sulphur (Suttle, 1974). The exact mechanism of this antagonism is not fully understood but it is hypothesised that the action is as follows:

During rumen fermentation dietary molybdenum and sulphur are altered to molybdate and hydrogen sulphide. These then form thiomolybdate. This complex binds the dietary copper with such strength that absorption is markedly reduced.

High dietary concentrations of inorganic iron salts (1-4 g/kg) have been shown to decrease the copper status of cattle (Coup & Campbell, 1964; Campbell et al., 1974; Humphries et al., 1983; Phillippo, Humphries & Garthwaite, 1987). The iron antagonism of copper is also sulphur dependant but little is known of the possible mechanism (Suttle, Abrahams & Thornton, 1984). High iron intakes by grazing ruminants are usually due to ingestion of soil contaminated pasture. In autumn and winter when pastures are more liable to poaching, herbage iron concentrations can increase from the normal 50-100 mg/kg DM to 4500 mg/kg DM (Winter, 1989).

Although high dietary iron concentrations can reduce the availability of copper causing reductions in liver and plasma copper concentrations, these changes often occur without clinical signs of deficiency. Studies at the Rowett Research Institute have shown that in cattle where an equal state of copper depletion was induced by either high molybdenum or high iron intake the clinical symptoms of reduced growth rate, changes in hair colour and infertility were only seen in those with molybdenum induced copper deficiency (Humphries et al., 1983; Phillippo et al., 1987; Bremner et al., 1987). In these studies the antagonistic effects of dietary iron were seen at concentrations of 500 mg iron/kg DM and in the latter study a dietary concentration of only 150 mg iron/kg DM was shown to deplete copper reserves after a period of 41 weeks. No additive effect of iron and molybdenum as antagonists of copper have been shown. However it has been suggested (Suttle, 1988) that there is a practical significance to consecutive antagonism by iron and molybdenum. This relates to outwintered animals which are subject to high dietary iron intakes as was established earlier. The copper reserves of these animals will become depleted by the start of the grazing season. They must then possibly face the further hazard of molybdenum

antagonism which will increase over the grazing season (Suttle et al., 1986) and lead ultimately to very severe copper deficiency complete with clinical signs.

To account for the interaction of molybdenum and sulphur on the copper requirements of animals these can be expressed as absorbable copper. For a specific diet the absorbability of dietary copper can be predicted from the equation of Suttle (1983b).

$$ACu = 5.72 - 1.297S - 2.785 \log_e Mo + 0.227 (Mo \times S)$$

where

ACu = predicted absorbability of copper (%)

S = sulphur, mg/kg DM

Mo = molybdenum mg/kg DM

From this the absorbable copper ingested can be calculated.

$$Cu\ abs = DCu \times \frac{ACu\ (F)}{100} \times I$$

where

DCu - dietary copper mg/kg

ACu - predicted absorbability of copper (%)

F - correction factor for hay or pasture

I - daily dry matter intake

Cu abs - absorbable copper ingested

The requirement of growing cattle for absorbable copper is shown in Figure A.

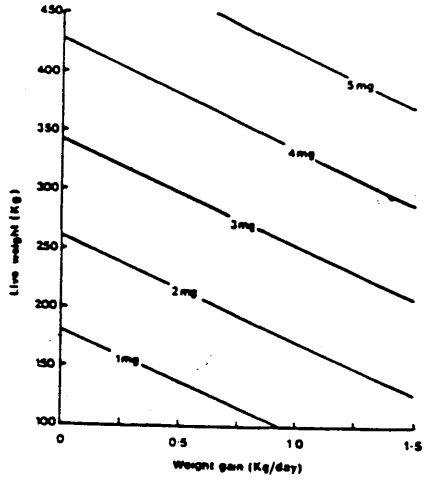



**Figure A Tentative estimates of relationships between body weight, daily weight gain and the requirement of cattle for absorbable Cu (mg/day) (SAC/SARI 1982).**

## Assessment of copper status

### Measurement of soil and herbage copper concentrations

As the previous section has detailed, the dietary concentration of copper has to be considered in association with the levels of dietary molybdenum, sulphur and iron.

The mean concentration of copper in British top soils is about 20 mg/kg DM with a wide range of values (2-2000 mg/kg) being observed (Bowie & Thornton, 1984). Soils derived from coarse grained arenaceous rock and acid igneous rocks contain lower concentrations of copper than those from fine grained sediments and basic igneous rocks (Thornton & Webb, 1970). The copper present in soils exists in a number of different forms not all of which are available to plants. This accounts for the fact that in most cases there is no direct relationship between soil and plant copper concentrations.

For Scotland, the soil associations and series have been classified according to the risk of molybdenum accumulation and risk of low copper concentrations in herbage (SAC/SARI, 1982).

The herbage content of copper, molybdenum and sulphur is influenced by the species composition, season and lime and fertiliser applications. On the same soil clover will have a higher copper content than ryegrass but it also has the ability to accumulate molybdenum. Ryegrass has a fairly low copper content. In one study over a six year period (Jarvis & Whitehead, 1981) the highest copper concentration recorded was 9.5 mg/kg and the mean concentration varied from 4.7-7.7 mg/kg DM.

Both molybdenum and sulphur show trends of increasing concentration in herbage over the grazing season from May to September. Copper concentrations also increase but to a lesser degree (Leech & Thornton, 1987).

Liming can have a major effect on the availability of copper to the grazing animal. Herbage copper concentrations show little or no change over a range of pH values but the herbage molybdenum concentrations can more than double when a soil pH increases from 5.5 to 6.5 (SAC/SARI, 1982).

Application of nitrogenous fertilisers such as ammonium nitrate which have an acidifying effect on the soil will therefore decrease the molybdenum concentrations. There is however a general diluting effect on all trace elements due to the increased growth which their application induces. Their effect on species composition may also influence trace element content of the herbage.

The dietary concentration of copper, molybdenum and sulphur is therefore modified by various factors.

The dietary copper allowance for cattle is 12 mg/kg DM intake (MAFF et al., 1983). This allowance was derived from ARC, 1980 which used a coefficient of absorption of 0.04 to calculate the requirement.

The dietary allowance acknowledges the effect of sulphur and molybdenum and using the equation of Suttle & McLaughlin, 1976 gives a table of coefficients of absorption for various levels of sulphur and molybdenum.

The data is shown in Table A.

**Table A** **Coefficient of absorption of copper as influenced by dietary molybdenum (mg/kg DM) and sulphur (g/kg DM) (From MAFF et al., 1983).**

| Dietary sulphur | Dietary molybdenum | | |
|---|---|---|---|
| | **1.0** | **3.0** | **5.0** |
| **1.5** | 0.052 | 0.047 | 0.043 |
| **3.0** | 0.038 | 0.032 | 0.027 |
| **4.5** | 0.028 | 0.021 | 0.016 |

**Measurement of liver and blood plasma copper concentrations**

Ruminant livestock have a high capacity for hepatic storage of copper and liver copper concentrations can be used as a good indicator of copper status. Low liver copper concentrations are characteristic of animals grazing copper deficient pastures (Wiener & Field, 1969) and copper supplementation can increase liver copper concentrations (Stoszek et al., 1986). Copper is fairly uniformly distributed within the liver of adult animals (Bingley & Duffy, 1972) but the difficulty in performing the biopsy on a sufficient number of animals to give a representative assessment of the group copper status is a major disadvantage. At post mortem it is however a useful diagnostic measure and the criteria suggested by the Scottish Veterinary Investigation Service (SVIS) for interpretation of liver copper concentrations is given in Table B.

**Table B SVIS assessment of copper status from liver copper concentrations.**

| assessment of status | liver copper mg/kg |
|---|---|
| adequate | **>30** |
| marginally deficient | **10-30** |
| deficient | **<10** |

Plasma copper concentrations can also be used as an indicator of copper status. About 95% of plasma copper is contained within the enzyme caeruloplasmin. There is a good relationship between plasma and liver copper status but only when animals are of a marginal copper status (Claypool et al., 1975; Stoszek et al., 1986). Above plasma copper concentrations of 9.4 umol/litre (0.6 mg/litre) the increases in liver copper concentration are not reflected by increases in plasma copper concentrations. Therefore above that level plasma copper concentrations do not adequately reflect the copper status of the animal. Values below 9.4 umol/litre are taken to be indicative of potential copper inadequacy since this equates to a liver copper concentration of below 30 mg/kg DM.

It has been shown that in situations of high dietary molybdenum plasma copper concentrations may seriously underestimate the copper status of the animal with up to 50% of the copper being unavailable (Dick, Dewey & Gawthorne, 1975; Smith & Wright, 1975; Humphries, 1980).

To accurately assess the copper status of a group of animals a representative number of animals should be sampled. Typically this would be around 10% although this may be insufficient in some cases (Tanner, Stednick & Leininger, 1988).

**Measurement of blood cuproenzymes**

Since many of the clinical symptoms of copper deficiency are related to the lowered activities of cuproenzymes there has been much interest in using enzyme activities to assess copper status. The use of blood enzymes has obvious advantages and the two main subjects of interest at present are caeruloplasmin and superoxide dismutase.

Caeruloplasmin is the major cuproenzyme in plasma and is synthesised within the liver. It has a half life of 3-5 days (Sternlieb et al., 1961) which means that its activity responds rapidly to changes in copper status. One advantage of its use is that only a small volume of plasma is required and that exogenous copper contamination has no effect. It also gives a measure of the biologically available copper. Unfortunately caeruloplasmin activities are raised in cases of infectious disease and parasitic infection.

Copper superoxide dismutase (SOD) activity is conferred upon the red blood cells at erythropoesis. The half life is much longer than that of caeruloplasmin and changes in SOD activity with copper depletion are slower than that of caeruloplasmin or plasma copper. It also shows good correlation to hepatic copper levels (Paynter, 1987). Lowered SOD activities are indicative of a severe prolonged copper deficiency. Approximate threshold levels for caeruloplasmin and superoxide dismutase are given in Table C.

**Table C Threshold levels of cuproenzymes, caeruloplasmin and superoxide dismutase.**

| | | |
|---|---|---|
| Caeruloplasmin | | **10** units/litre whole blood |
| Superoxide dismutase | calf | **0.5** mg SOD/g Haemoglobin |
| | adult | **0.3** mg SOD/g Haemoglobin |

## SELENIUM

### Function

Selenium is an integral component of the enzyme glutathione peroxidase (GSHPx) with 4 g atoms of selenium per mole of enzyme (Flohe, Gunzler & Schock, 1973). GSHPx catalyses the breakdown of hydrogen peroxide and organic hydroperoxides produced by glutathione during redox cycling. It thus acts as part of the cells defence system against oxygen induced damage and it is within this antioxidant system that the essential nutrients selenium and Vitamin E are associated. Their relationship is shown in Figure B.

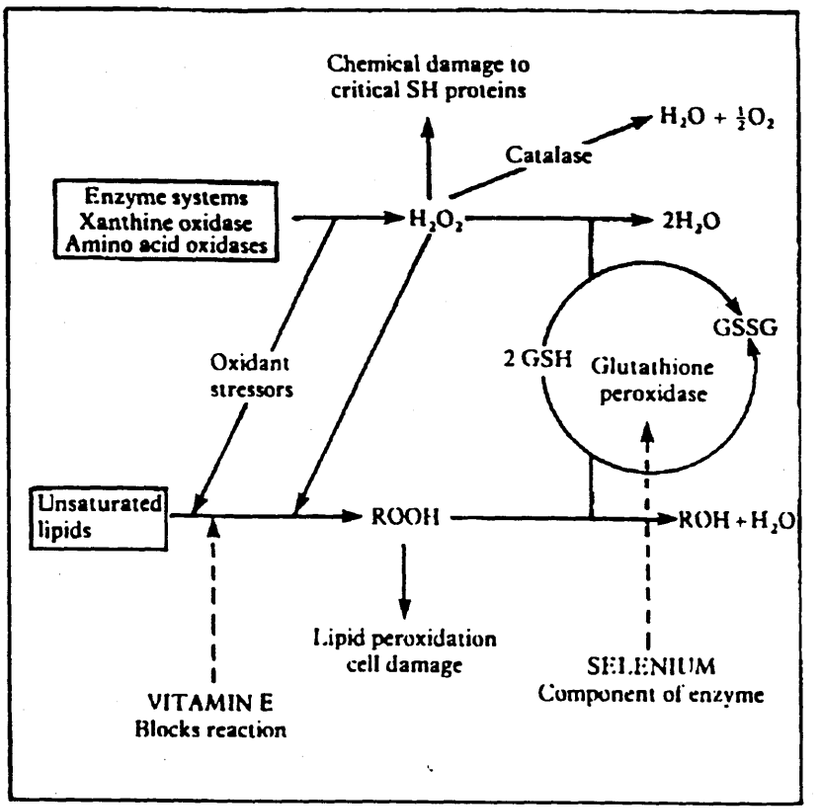



**Figure B. The relationship between selenium and Vitamin E. (Putnam & Combden, 1987).**

Inadequate levels of selenium and or Vitamin E can result in cell damage. Because of their association many of the deficiency diseases and selenium responsive syndromes only respond to supplementation with both of these nutrients.

## Deficiency symptoms and responsive disorders

### Nutritional myopathy

This is the most commonly observed clinical syndrome of selenium deficiency. It is an acute myodegenerative disorder in which the pale colour and often white striation of affected muscle explains the more common name of 'white muscle disease'. Skeletal and cardiac muscles are affected but the major clinical signs involve mainly skeletal muscle. Nutritional myopathy generally occurs in animals between one and four months of age. Affected animals are listless, walk reluctantly and with a stiff gait. This is followed by prostration and death. It can be of rapid onset especially after exertion. It is most commonly seen in the spring with suckling calves shortly after transfer to grass. Increased polyunsaturated fatty acids (PUFA) intake from spring grass and the period of excitement involved in transfer contribute to the appearance of clinical signs. In such cases sudden death may occur due to focal myopathy occurring in the heart muscle.

There are reports of a similar syndrome in bucket reared dairy calves where sudden death occurs around the time of milk feeding. (Cawley & Bradley, 1978). Supplementation at birth with selenium and Vitamin E prevented further incidence.

In sheep a congenital type of nutritional myopathy occurs (Combs & Combs, 1986) where lambs are stillborn or die within a few days of birth. Again a period of physical exertion is often involved.

In cattle the involvement of selenium/Vitamin E deficiency in the weak calf syndrome (Stauber, 1976) where calves are born alive but severely depressed and generally die after a few days has been subject to much debate. (Taylor, Puls & MacDonald, 1979; Rice et al., 1986; Cawley 1987; Logan et al., 1990; Sluijter, Zimmer & Wouda, 1990). As yet no definite link has been established.

During the 1970's there were many reports of nutritional myopathy in yearling cattle. (Christl, 1971; Allen et al., 1975; Anderson, Berrett & Patterson, 1976; McMurray & McEldowney, 1977). This was a delayed and more diffuse myopathy with myoglobinuria shown but with some animals recovering spontaneously. Prior to these reports the disease was considered to be restricted to suckling and bucket reared calves. The aetiology and pathology of this syndrome is more complicated than that in younger animals. It has been shown (Arthur, 1979; Siddons & Mills, 1981) that some animals maintained on a low selenium/Vitamin E diet for long periods of time show no symptoms of the disease. It is therefore thought that selenium/Vitamin E deficiency predisposes older animals to nutritional myopathy but that other trigger factors are involved, such as exercise, stress and high PUFA intakes.

**Reproductive disorders**

Placenta retention is seen commonly in most dairy herds (Hidiroglou, 1982) where premature birth, abortion and difficult births result in a 'normal' incidence of 5-10%. If the incidence exceeds 15% then the possibility of a nutritional component to the problem should be considered (Ward, 1981).

In cattle the placenta is normally expelled 2-8 hours after parturition with a placenta being considered to be retained if expulsion has not occurred within 12 hours. In cases where the placenta is retained there is an increased incidence of uterine infection with metritis occurring in more than 50% of such cases (Joosten, Stelwagen & Dijkhuizen, 1988). Retention of the foetal membranes therefore indirectly delays oestrus and decreases fertility (Britt, Cox & Stevenson, 1981). Such effects can have major economic significance with a retained placenta incidence of 30% costing the dairy farmer an estimated £22/cow/year (Joosten et al., 1988).

The incidence of retained placenta in dairy cows has been shown to be related to the selenium status of the herd (Trinder et al., 1969; Trinder, Hall & Renton, 1973; Julien et al., 1976; Eger et al., 1985). Supplementation with selenium/Vitamin E can reduce this problem. In one study (Trinder, Woodhouse & Renton, 1969) an injection of a selenium preparation and Vitamin E four weeks before calving reduced the incidence of retained placenta from 42% to zero.

Selenium supplementation has also been shown to reduce the incidence of cystic ovarian disease (Harrison, Hancock & Conrad, 1983) which can result in cows becoming anoestrus.

Since both these selenium responsive conditions can affect cow fertility it is not surprising that improvements in dairy cow fertility have been reported when deficient herds have been supplemented with selenium (McClure, Eamens & Healy, 1986; Tasker et al., 1987; MacPherson et al., 1987; Ehret et al., 1989). The effects of selenium supplementation on conception rate to first service were quite dramatic with one study showing an improvement from 30% to 58% (McClure et al., 1986).

**Unthriftiness**

A subclinical condition has been described where reduced growth rate and sometimes diarrhoea is associated with selenium deficiency. (Andrews, Hartley & Grant, 1968; Langlands et al., 1989). Cattle suffering from such a condition have been shown to respond to selenium suplementation with increases in liveweight gain of up to 0.31 kg/day (Hathaway et al., 1979; Gleed et al., 1983). In general however the syndrome is not as severe as that observed in sheep (Oldfield, Schubert & Muth, 1963; Blaxter, 1963; McDonald, 1975).

## Assessment Of Selenium Status

### Selenium concentration in soil and herbage

The principal factor in determining the selenium status of the grazing ruminant is the selenium content of the herbage which it consumes. In turn the herbage content is influenced by the concentration of selenium in the topsoil in which it grows, although the relationship is not always a direct one (Hartfiel & Bahners, 1988). Comprehensive maps of soil selenium content such as those for copper and cobalt (SAC/SARI, 1982) are not available and there is very little knowledge of content and availability of selenium in soils. A recent survey of surface soils in England and Wales showed a mean selenium content of 0.48 mg/kg DM with a range of 0.01-4.66 mg/kg (Dorst Van et al., 1985). Those soils with calcareous and coarse grained sedimentary parent materials had the lowest levels and peaty soils showed the highest levels.

The uptake of selenium by plants is determined by factors which influence the water solubility of selenium (Combs & Combs, 1986). Soil pH is the major factor with the availability of selenium to plants increasing as soil conditions become more alkaline (Davies & Watkinson, 1966). This effect is substantially greater in sandy soils than in loams (Gissel-Neilson, 1971).

The use of fertilisers can also affect the selenium content of herbage. As with copper the increased growth in herbage which results from fertiliser application can have a 'diluting effect' with the selenium concentration of the herbage being reduced (Burridge, Reith & Berrow, 1983). The use of some fertilisers which lower soil pH (eg ammonium sulphate) may also reduce the availability of the selenium in the soil to the herbage.

The Agricultural Research Council (1980) concluded that the minimum selenium requirement was 0.05 mg/kg DM intake. However, the recommended dietary allowance for selenium was increased to 0.1 mg/kg DM (MAFF et al., 1983). Selenium concentrations greater than 0.06 mg/kg DM are required to prevent white muscle disease (Oldfield et al., 1963; Gardiner & Gorman, 1963). The other selenium responsive disorders are also unlikely to occur with dietary intakes above that level (SAC/SARI 1982). It has however been demonstrated that 0.11-0.12 mg/kg DM is required to maintain the tissue and blood GSHPx activity (Underwood, 1981a).

Measurement of soil and herbage selenium concentrations is therefore one method of assessing the selenium status of the grazing animal and will give an indication of the risk of selenium deficiency.

**Blood selenium concentrations and glutathione peroxidase activity**

Whole blood and plasma selenium concentrations can be used to provide an index of the selenium status of the grazing animals (Langlands et al., 1989). Selenium analysis of biological samples is however laborious and expensive. An easier method is to measure at $37^0$C the whole blood activity of the selenoenzyme glutathione peroxidase (GSHPx). The activity of GSHPx has been shown to be highly correlated to blood selenium concentrations (Anderson, Berrett & Patterson, 1978; Koller et al., 1984; Counotte & Hartmans, 1989) and is at present the preferred indicator for assessment of selenium status.

The GSHPx activity of blood is due to the enzyme being present within the cell membrane of the erythrocytes. Since 90% of the GSHPx activity can be attributed to erythrocytes the whole blood activity is expressed in units per ml of packed cell volume (PCV). Erythrocytes have a life span of about 120 days and their GSHPx activity is determined at synthesis and cannot be altered subsequently. Therefore animals should have been consuming the same diet for at least 3 months for a correct assessment to be made.

The criteria used by the Scottish Veterinary Investigation Service for assessment of selenium status is shown in Table D.

**Table D** **SVIS assessment of selenium status from whole blood glutathione peroxidase activity.**

| assessment of status | whole blood GSHPx activity (iu/ml PCV) |
|---|---|
| adequate | **>17** |
| marginally deficient | **8-17** |
| deficient | **<8** |

Animals with whole blood GSHPx activities within the deficient range are only at risk of nutritional myopathy and need not show clinical signs (Arthur, 1979).

If there are clinical signs and muscle damage is suspected then the blood plasma activity of creatine phosphokinase (CPK) can be measured. This enzyme is a normal component of muscle cells and if damage to them has occurred it leaks out into the plasma. Normal CPK levels are less than 100 units per litre with levels of 5-10,000 units per litre being seen in cases of nutritional myopathy (Anderson et al., 1976; Rice & McMurray, 1986; Arthur, 1988).

The selenium status of the grazing ruminant can therefore be assessed by measurement of whole blood GSHPx activity. Examination of the status of several animals would be expected to give a more reliable estimate of the problem within the herd.

# COBALT

## Function

The active form of cobalt within the animal body is cobalamin or Vitamin $B_{12}$. This compound cannot be synthesised by animal tissue and the requirement of the ruminant is for the preformed cobalamin. Rumen micro-organisms are capable of converting dietary cobalt to cobalamin and other cobalamides which are non-active (Bigger, Elliot & Richards, 1976). The symbiotic relationship between these mircro-organisms and the ruminant provides it with a source of Vitamin $B_{12}$. The efficiency of conversion of dietary cobalt to tissue stores of Vitamin $B_{12}$ is however very poor (Hedrich, Elliot & Lowe, 1973).

The main function of Vitamin $B_{12}$ is in propionate metabolism as an enzyme cofactor to the mitochondrial enzyme methylmalonyl coenzyme A mutase. This is responsible for the conversion of methymalonyl coenzyme A to succinyl coenzyme A which can then enter the tricarboxylic acid cycle. A deficiency of Vitamin $B_{12}$ limits the conversion of methylmalonyl coenzyme A and thus limits propionate metabolism. Since ruminants rely heavily upon this for an energy source this explains the severe effects seen in cobalt deficient animals.

A second Vitamin $B_{12}$ dependant reaction is the conversion of homocysteine to methionine. A reduction in that may account for the poor nitrogen retention seen in some cases of Vitamin $B_{12}$ deficiency (Underwood, 1981b). Through its effect on methionine, Vitamin $B_{12}$ deficiency can result in the depletion of liver folate reserves resulting in increased secretion of formiminoglutamate (Gawthorne, 1968).

## Deficiency symptoms and responsive disorders

Clinical cobalt deficiency generally occurs after animals have been grazing cobalt deficient pasture for some time (Underwood, 1981) due to the time taken for liver Vitamin $B_{12}$ stores to become depleted. The initial sign of deficiency is gradual loss of appetite, thought to be due to the increased blood propionic acid levels (Marston, Allen & Smith, 1961). A rough coat and nervousness may also be observed as well as a depraved appetite.

Severe deficiency results in an emaciation which is indistinguishable from starvation and except for the anaemia which is also shown. Unless preventive action is taken death may occur. Offspring from cobalt deficient animals are often weak at birth and have reduced chances of survival. Effects on milk yield and fertility have been observed but these can probably be linked to reduction in appetite. Symptoms of cobalt deficiency are however more likely in young growing animals when the cobalt requirement is greater.

## Assessment of cobalt status

### Measurement of cobalt concentration in soil and herbage

Survey results show that the mean cobalt content of Scottish soils is 11.9 mg/kg with a range of 2.1-88.0 mg/kg (Berrow & Ure, 1985). Cobalt deficient soils and herbage are likely where the soil parent material is acid igneous, sandstone or other arenaceous material.

The uptake of cobalt from the soil is reduced with increasing pH (Mitchell, 1972) and improved natural drainage (Berrow & Ure, 1985). Large differences between sward species are seen with white clover having a higher concentration than ryegrass and ryegrass in turn having a higher concentration than timothy. The stage of maturity and season can substantially alter this situation (Klessa, Dixon & Voss, 1989). A mature timothy grass sward is most likely to be of low cobalt status and hence timothy hay is often used as a major component of experimental cobalt deficient diets (Fisher & MacPherson, 1990; Paterson & MacPherson, 1990). Application of fertiliser nitrogen has been shown to increase the cobalt concentration of herbage by 100% and in so doing elevate deficient herbage to adequate status (Klessa et al., 1989). Part of this effect may be due to the acidifying nature of some such fertilisers.

The dietary allowance for cobalt is 0.11 mg/kg DM intake (MAFF et al., 1983) with herbage containing less than 0.08 mg/kg DM being regarded as deficient. However the critical value for cattle is often taken as being lower at 0.04 mg/kg DM (Sherrel, McIntosh & Brunsden, 1989). Since soil cobalt concentrations are much greater (up to 88 mg/kg DM) than herbage values, soil contamination can easily cause misleadingly high herbage cobalt concentrations.

The criteria for assessment of soil and herbage cobalt status are given in Table E.

**Table E. Assessment of cobalt status from soil and herbage cobalt concentrations (Berrow & Ure, 1985, SAC/SARI 1982).**

| assessment of status | soil cobalt mg/kg DM | herbage cobalt mg/kg DM |
|---|---|---|
| adequate | >15 | >0.10 |
| marginal | 5-15 | 0.08-0.10 |
| deficient | <5 | <0.08 |

**Measurement of liver and blood Vitamin B12 concentration**

Liver concentrations of cobalt and Vitamin $B_{12}$ are useful indicators of cobalt status (Mertz, 1987). The Vitamin $B_{12}$ concentration is a better indicator of cobalt deficiency since under that circumstance the levels fall faster than cobalt concentrations and oral dosing of cobalt can increase liver cobalt status without affecting ruminal Vitamin $B_{12}$ synthesis (SAC/SARI, 1982).

Plasma Vitamin $B_{12}$ concentrations can also be used to diagnose cobalt deficiency. As cobalt intakes fall so decreases in plasma Vitamin $B_{12}$ concentrations are seen (Sherrel, Brunsden & McIntosh, 1987). However starvation and yarding (Clark & Millar, 1983; Millar, Albyt & Bond, 1984) can cause spuriously high plasma Vitamin $B_{12}$ concentrations and inactive analogues (Price, 1989) can interfere with the assay. Inconsistencies between laboratories and analytical techniques have also been noted (Mollin et al., 1980; Slater et al., 1985). Further problems are caused by the fact that in housed animals fed a cobalt deficient diet low Vitamin $B_{12}$ concentrations ($<50$ ng/l) were observed for at least 25 weeks before liveweight gain reductions were noted (Paterson & MacPherson, 1990) observations indicate that this effect is common in cattle at grass (SVIS, 1990c). The assessment of cobalt status from plasma Vitamin $B_{12}$ concentrations as used by the Scottish Veterinary Investigation Centre is shown in Table F.

**Table F** **SVIS assessment of cobalt status from plasma Vitamin $B_{12}$ concentrations.**

| assessment of status | plasma Vitamin B12 (ng/litre) |
|---|---|
| adequate | >200 |
| marginally deficient | 150-200 |
| deficient | <150 |

Due to problems associated with the determination of concentrations of plasma Vitamin $B_{12}$, the measurement of methylmalonic acid (MMA) has been suggested as an alternative diagnostic aid. MMA accumulates during the disturbances to propionate metabolism. Encouraging results were seen with urinary MMA in assessing cobalt status (Quirk & Norton, 1988) but obvious problems in obtaining samples make it difficult to adopt this procedure. The development of a method for plasma MMA determination (McMurray et al., 1986) gave the technique more scope. A recent study has shown that plasma MMA has promise as a diagnostic tool but at present, use of both Vitamin $B_{12}$ and MMA provide the best assessment (Paterson & MacPherson, 1990). Suggested criteria for assessment are: normal <2 umol MMA/litre; subclinical 2-4 umol MMA/litre and deficient >4 umol MMA/litre.

## Methods for the supplementation of grazing cattle with trace elements.

### Introduction

When a suspected deficiency of one or more trace elements has been revealed by either routine blood sampling or as the result of a specific clinical investigation then the decision must be made as to whether supplementation is economically justified. If the deficiency is revealed by the latter route, it is likely that a specific problem such as disease, increased mortality, poor growth rate and poor fertility was being investigated. In these circumstances the decision is more easily made than if there is only a blood profile on which to make the decision.

Depending upon the severity of the problem it may be possible to increase the trace element status by supplementation during the winter feeding period by incorporation into the concentrate part of the ration. This may be appropriate for copper and selenium where there is some body storage but not for cobalt where a continuous supply to the rumen is required for the microbial synthesis of Vitamin $B_{12}$.

Should this not be possible or the deficiency be more severe, then some form of supplementation to the grazing animal must be considered. There are a range of methods available for trace element supplementation and these can be divided into six main groups as follows:

1. Pasture application
2. Free choice minerals
3. Drinking water
4. Oral dosing
5. Parenteral
6. Slow release technologies

**Pasture application**

A simple method to supplement the diet of grazing cattle is to increase the trace element content of the herbage. This may be done by either increasing the availability of existing soil levels of these elements to the plants, for example by altering soil pH, or by direct application of trace elements to the pasture.

Providing that the compound chosen is relatively cheap then this method can be cost effective since the trace element can be applied with other routine treatments such as superphosphate. This method also avoids handling the animals and thus saves on labour costs. It does not however have any application in extensive systems where large areas of land would be required to be treated.

Copper has been applied to pasture to improve plant and animal performance for over 100 years (Gilkes, 1981). The most commonly used compound is copper sulphate but a wide range of other copper compounds have been utilised (Delhaize, Loneragan & Webb, 1987). Application of 6 kg copper per hectare can increase herbage copper concentrations from 4.3 to 7.4 mg/kg DM (Burridge et al., 1983). On certain soil types such supplementation gives long term residual activity with a single treatment remaining effective for up to ten years (Langlands, 1987).

The main disadvantage of this method of copper supplementation is that it has limited effectiveness against deficiencies induced by molybdenum-sulphur antagonism.

Selenium can also be applied in this manner. Sodium selenate encapsulated in a water soluble prill to offset toxicity problems can be used at a rate of 10 g selenium per hectare (Watkinson, 1983; Halpin et al., 1985). Such application results in a rapid increase in herbage selenium concentrations but these soon fall and the treatment is generally effective for only one year (Halpin, Hanrahan & McDonald, 1987; Langlands, 1987; Watkinson, 1989). This short term effectiveness and the high material costs are the main disadvantages of this system.

One possible method of reducing the cost is to apply the selenium to only a small area of each field, for example, a strip up the middle of the field. Alternatively several groups of animals could consecutively graze a single field to which the selenium had been applied (Millar & Meads, 1987).

Cobalt sulphate application can be used to increase pasture cobalt levels. A single application of 0.5 kg cobalt per hectare has been shown to maintain adequate pasture levels for four years (Burridge et al., 1983). A higher level of 2 kg cobalt per hectare is recommended and will provide adequate levels for 3-5 years (Scottish Agricultural Colleges and the Macauley Soil Research Institute, 1985).

High soil pH will however decrease the response (Klessa et al., 1989) and this may account for the fact that in some cases up to 6 kg per hectare have been required to maintain adequate pasture levels (McLaren et al., 1979).

**Free access minerals**

Trace elements are commonly added to mineral mixtures for free choice feeding. The mixture can be a powder, a block or incorporated into molassed liquids. This method of supplementation is generally considered to be unreliable and to be used only as a last resort when no other method is practically feasible (MacPherson, 1983). The quantity consumed by individuals will be extremely variable and cannot be predicted in advance (Kendall, 1977; Sword et al., 1984; Money, Meads & Morrison, 1986). In one study the daily intake of a mineral mixture by suckler cows varied from zero to 500 g with more than 50% of the animals receiving inadequate amounts (Hemingway, 1982).

When this method was used to supplement cows with copper by providing a mineral containing 2500 mg/kg the mean blood plasma copper concentration was only increased from 0.43 to 0.59 mg/litre over the 150 day trial period and over 40% of the animals remained deficient.

Palatability of the mixture, protection from the weather, animal training and the number and location of the feeding points can considerably improve the situation but variation in animal intake makes it an unacceptable method of supplementation.

**Water supplementation**

This provides another method of supplementation which does not involve the handling of individual animals. It does however require that the sole source of water is a trough and this probably limits the use to more intensive systems where cattle are grazing enclosed pasture.

As with free access supplements the trace element intake via water will be subject to variation. Within a group of animals, individual's intake of water show differences of up to 50% (Kelley, 1945; Thompson, Henry & Kon, 1964). For dairy cattle at grass individual water intakes have been shown to vary from 6 to 52 kg/cow (Campbell, 1958) and 4 to 44 kg/cow (Castle, 1972). The intake of the group as a whole will be influenced by rainfall, maximum air temperature and dry matter content of the herbage (Castle & Watson, 1973; Castle & Watson, 1975).

However unlike free access supplements each animal will have a minimum daily intake of water. For first season grazing calves this has been estimated at 2.3 litres/head (O'Shea & Downey, 1981). This fact has been utilised in the provision of anthelmintics via the drinking water (Downey & O'Shea, 1977) where the required dose is added to the minimum intake for each calf. This attempts to ensure that variation in intake is minimised.

Supplementation of the water supply of dairy cows with copper by adding copper sulphate twice daily to the water trough has been shown to give increases in plasma copper concentration equal to that achieved by giving a copper glycinate injection containing 120 mg copper (Smith & Moon, 1976). The copper concentration of the water was such that the daily intake was estimated at 178 mg copper per head. This level of copper supplementation gave a significantly greater increase in liver copper levels than seen with the copper injection (143 v 44 mg copper/kg DM) and this was achieved by supplementing the water for only 60 days. The authors noted that there was a danger of excessive liver copper storage should such high levels of supplementation be continued.

With calves given 2-3 mg copper per litre there was also a rapid response to supplementation via the drinking water (Humphries, 1980). The calves had a molybdenum-induced copper deficiency and were showing the typical signs of scouring and high levels of TCA insoluble copper in the blood plasma. Within three days of commencement of supplementation the scouring had ceased and within five days the plasma copper insoluble in TCA had fallen from 65% to zero.

These previous trials had relied on the addition of copper to the water supply by hand on a daily basis. This was obviously a labour intensive method and would probably not be feasible on a farm scale. The development of an automatic

metering device (Humphries, 1978) made water supplementation a more feasible method of providing trace elements. The device has an elevated reservoir which delivers the liquid trace element supplement to the water trough. It operates from any piped water supply and is therefore suited to grazing or housed animals.

When suckler cows received copper-supplemented water from the device with a concentration of 2.5 mg Cu/litre this was sufficient to prevent an end-of-season fall in their plasma copper concentrations and at housing these were significantly greater than the control group. Similarly where water supplemented with 2-3 mg Cu/litre was provided to growing cattle grazing 'teart' pasture this was sufficient to prevent scouring in these animals (Humphries, MacPherson & Farmer, 1983). Withdrawal of copper-supplemented water resulted in the onset of severe scouring. Supplementation with copper via the metering device has also been shown to be almost as effective as repeated parenteral treatment (Humphries et al., 1983).

Cobalt as cobalt sulphate at 0.23 mg Co/litre supplied by the metering device to grazing heifers and housed calves is sufficient to increase plasma Vitamin $B_{12}$ concentrations (Humphries, 1980).

An alternative method of adding trace elements to the water supply is available. Soluble tablets with formulations containing copper, cobalt and selenium can be used ('Aquatrace'). These are suspended in a plastic container within the water trough and the matrix is specially formulated to ensure that a constant level of trace element is maintained within the water.

This method has generally proved to be less effective due to insufficient quantities of the trace element being released. This may be due to the formation of a sludge at the bottom of the trough (Farmer, 1983; Suttle, 1983b). Analysis of the sludge in one trial showed a copper concentration of 976 mg/kg (MacPherson, 1983).

**Oral dosing**

Giving trace elements in solution by oral dosing is a long practiced method of supplementation (Jamieson & Allcroft, 1950). It ensures that each animal gets the desired quantity of trace element and since it used the salts of the required trace elements dissolved in water raw material costs are not expensive. However, as with other methods which involve individual treatment of the animals there is a cost involved in gathering and handling.

If this operation can be carried out at the same time as other routine tasks then this cost may be reduced provided such diverse treatments are not contra-indicated. Incorporation of the trace element into anthelmintic drenches is possible and this would be a cost effective method (Killen, 1987; MacPherson, Rice & Paterson, 1987; Field et al., 1988) but there are problems in achieving homogenity and special formulations may be necessary (Harvey, 1989). Such problems have resulted in cases of selenium poisoning in lambs (Anderson, Berret & Parker, 1985; Hopper, Greig & McMurray, 1985).

Copper supplementation by this method is usually in the from of copper sulphate and 1.5 g of copper per head in this form given at monthly intervals has been shown to increase the growth rate of young cattle but no difference in plasma copper levels were noted (MacPherson, 1983). However with beef suckler cows, dosing with similar levels of copper during the last 3 months of gestation resulted in higher plasma copper concentrations in the calves (MacPherson, Voss & Dixon, 1979). This capacity for placental transfer is well developed in the bovine (Suttle et al., 1980) and provides a useful but temporary means of supplementing the calf.

Both sodium selenate and sodium selenite are used for oral dosing. Dose rates of 0.1-0.2 mg selenium/kg liveweight given as a single treatment have proved to be effective in increasing blood status, liveweight gain and fertility in deficient cattle (Davis, 1974; Morton, 1981; Ehret et al., 1989).

Cobalt sulphate can be used to supply cobalt by oral dosing. Dose rates of 5-20 mg cobalt daily (Bal & Dwarkanath, 1989), 100 mg fortnightly (MacPherson, 1983) or 350 mg as a single dose (Reid, 1981) have been reported. Responses to supplementation in the form of plasma Vitamin $B_{12}$ and liveweight gain were recorded only for the more frequent treatments. This is in line with the much greater number of reports of cobalt supplementation in lambs which shows that plasma Vitamin $B_{12}$ levels peak 3-5 days after oral dosing with cobalt but return to their initial level by around 21 days after dosing (MacPherson et al., 1987; Bremner et al., 1988; Field et al., 1988; MacPherson, 1989; Suttle et al., 1989; Suttle et al., 1990.

**Parenteral supplementation**

This method overcomes some of the problems associated with oral methods. Less frequent doses and known quantities of trace elements are given to each animal. Much lower quantities of trace elements are required to be effective. For example, 50 mg of copper as copper sulphate given intravenously is equal to 500 mg of copper in the same form when given orally. This is due to the bypassing of intestinal interactions and means that more than 75% of a parenteral dose can be stored in the liver compared to less than 10% of an oral dose (Allen, 1987).

Intravenous administration of large numbers of cattle is obviously not possible so parenteral preparations have been formulated for intramuscular and subcutaneous injection. Choosing such preparations suitable for parenteral use involves achieving a balance between the efficacy and toxicity of the compounds (Suttle, 1981).

For copper supplementation, a list of those compounds available in the United Kingdom is given in Table G. A single injection of copper-calcium edetate (100 mg Cu) can increase hepatic copper stores such that they are significantly higher than untreated animals for up to 200 days after treatment (Langlands et al., 1989). More often, repeated injections are required to maintain adequate plasma copper levels especially in cases of severe deficiency (Thornton, Kershaw & Davie, 1972; Gleed et al., 1983; Rogers & Poole, 1988).

Another problem associated with parenteral copper supplementation is the risk of toxicity. With some copper edetate and other compounds death can occur when only four times the therapeutic dose is given (Bohman et al., 1987).

Problems of reactions at the injection site with abcesses, local irritation and discolouration have been frequently noted (Ammerman, 1970; Bell et al., 1976; Deland et al., 1979; Gomm, Weswig & Raleigh, 1982).

With parenteral selenium supplementation the problem of toxicity is much greater because of the low therapeutic index of selenium. The LD 50 for sheep of sodium selenite for example is only 0.45 mg/kg liveweight. Three compounds are available for use in the United Kingdom and details are given in Table H. Two of the proprietary injections also include Vitamin E in recognition of the close link between the two substances.

For selenium as sodium selenite, 30 mg Se given every three months is required to maintain the status of adult cattle and 10 mg Se for calves (Andrews, Hartley & Grant, 1968). In cases of severe deficiency however monthly injections with 20 mg Se as selenite may be required (Hidiroglou, 1989)

A similar situation exists when selenium is given in the selenate form with growing cattle requiring two injections of 0.15 mg Se/kg liveweight to maintain adequate status throughout the grazing season.

These problems of repeated injections being required prompted Knuttler, Marble & Blincoe, (1961) to use an oil-beeswax mixture for the suspenion of barium selenate. The purpose of this viscous excipient was to reduce the rate of release of selenium from the injection site and thus provide a subcutaneous depot of the trace element. Barium selenate is itself less soluble than the sodium or potassium salts and this allows dose rates of 1 mg/kg to be injected with safety.

This large dose and the slow release provide increases in blood glutathione peroxidase activities which persist for 6-12 months (MacPherson et al., 1987; Tasker et al., 1987).

Concern has however been expressed at the high levels of selenium found at the injection site and the potential toxicity of those levels in meat used for human consumption (Mallinson, Allen & Sansom, 1985).

Parenteral cobalt supplements are of little or no value since the requirement of the animal is for a continuous supply of cobalt within the rumen for Vitamin $B_{12}$ synthesis and that there is no easy route for tissue cobalt to return to the rumen (Mertz, 1987). A subcutaneous Vitamin $B_{12}$ injection is available with a dose of 1-4 mg of cyanocobalamin being used to correct Vitamin $B_{12}$ deficiency (Judson et al., 1982). However plasma Vitamin $B_{12}$ concentrations have been shown after a rapid increase, to return to original levels within 24 hours (Schultz & Judson, 1985).

**Table G Copper compounds for parenteral administration to cattle.**

| compound | mg Cu/ml | administration | dosage | notes |
|---|---|---|---|---|
| Copper calcium edetate | 50 | subcutaneous | 1 ml <18 months<br>2 ml >18 months | the dosage may be required to be double to be effective |
| Copper methionate | 20 | intramuscular | 2-5 ml young cattle<br>6 ml adult | some local reaction may occur |
| Diethylamine copper oxyquinoline sulphonate | 6 | subcutaneous | 2-8 ml <250 kg<br>10 ml >250 kg | administer 4 weeks after turnout and then 6 months later |



**Table H Selenium compounds for parenteral administration to cattle.**

| compound | mg Se/ml | mg Vit E/ml | administration | dosage | notes |
|---|---|---|---|---|---|
| Barium selenate | 50 | | subcutaneous | 1 ml/50 kg | reaction at injection site may occur |
| Potassium selenate | 1.5 | 68 | subcutaneous/ intramuscular | 1-2 ml/45 kg | if required repeat after 2-4 weeks |
| Sodium selenite A | 2.5 | | subcutaneous/ intramuscular | 2 ml/100 kg | administer at three-monthly intervals |
| Sodium selenite B | 0.5 | 150 | intramuscular | 1 ml/50 kg | for therapy 1 ml/30 kg |

**Slow release technologies**

A number of these are available for trace element supplementation. The basic strategy is that the object containing the trace element lodges within the gastrointestinal tract, usually the reticulo-rumen. Density and particle size are important in ensuring retention. From there it releases trace elements over an extended period of time.

(i) Intraruminal pellets
(ii) Copper oxide needles
(iii) Soluble glass bolus

**Intraruminal pellets**

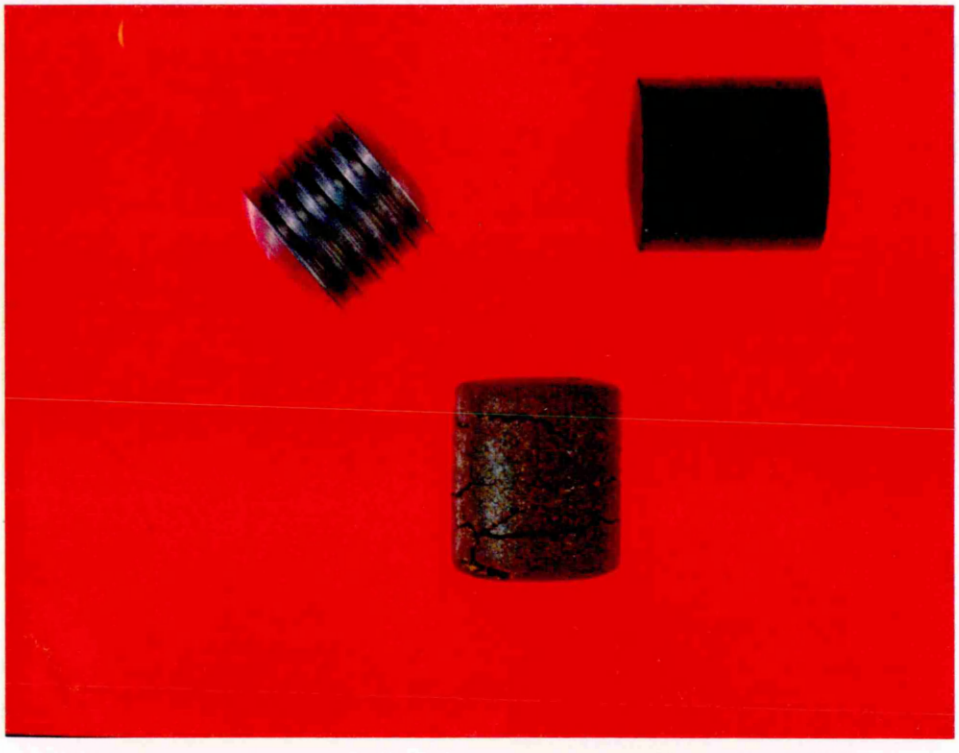
These are small pellets of iron to which initially cobalt oxide (Dewey, Lee & Marston, 1958) and later elemental selenium (Kuchel & Buckley, 1969) have been added. They are administered using an oesophageal balling gun and because of their density (approximately 5 $g/cm^3$) they lodge in the reticulo-rumen where they release either cobalt or selenium over a prolonged period. Thus each individual animal is required to be handled but a single treatment will provide an adequate supply of trace elements for the grazing season or longer.

For the supplementation of cattle with cobalt they have a further advantage in that a continuous supply is provided direct to the site of Vitamin $B_{12}$ synthesis.

The use of the cobalt 'bullet' as it is more commonly known has however met with mixed success. Favourable responses in plasma Vitamin $B_{12}$ concentrations and liveweight gain have been recorded (Sherman et al., 1959; Fearn, 1961; MacPherson, 1981; Nicol et al., 1983). However almost an equal number of reports show no response occurring (Essig, 1962; Alexander et al., 1967; Cole, Murphy & Poole, 1979; MacPherson & Dixon, 1980). This may relate to the cobalt bullet becoming coated with calcium phosphate (Poole & Connolly, 1967) which causes a rapid decline in the rate of release of cobalt into the rumen (Allen et al., 1988). It is recommended that either a second bolus or a grub screw be adminstered to provide abrasion and prevent the coating forming. The circumstances leading to the development of this coating have not been described. Plate 1 shows the cobalt bullets and grub screw suitable for administration to sheep and the degree of coating often observed. Plate 2 shows the same products for cattle.

**Plate 1.**
**A cobalt bullet and grinder suitable for administration to sheep. The other three bullets show the degree of coating which can occur.**

**Plate 2.**
**A cobalt bullet and grinder suitable for administration to cattle. The other bullet was recovered at slaughter and shows signs of rusting and fracturing.**



The selenium pellet for cattle weighs 30 g and consists of 95% iron and 5% elemental selenium. One pellet is sufficient to increase and maintain blood glutathione peroxide activities for 4-6 months for most classes of stock (MacPherson & Chalmers, 1984).

As with the cobalt bullet, concern over coating has led to the administration of a grub screw or an additional pellet. When two pellets are given, the period of increased blood glutathione peroxidase activities may be extended to 18 months (Judson et al., 1982; Judson & McFarlane, 1984). The selenium supplied in this manner can protect calves born to treated cows from nutritional myopathy (Hidiroglou, Proulx & Jolette, 1985).

In addition to the problem of coating, regurgitation of the selenium pellet has been reported (Koh & Judson, 1987).

**Copper oxide needles**

These are rod-shaped particles of oxidised copper wire usually administered in a gelatin capsule and given to the animal via an oesophageal balling gun. The density, size and shape of the particles is such that they are retained. In cattle the particles lodge within the reticulo-rumen and the abomasal folds (Suttle & Valente, 1981). The copper is released in the acid environment of the latter (Dewey, 1977). The half life of the particles within the gastrointestinal tract is between 50 and 172 days (Suttle, 1987; Costigan & Ellis, 1980; Deland et al., 1986). The fact that the half life decreases with increasing dose indicates that there is a limit to the amount of particles which can successfully be retained. Observations that increasing doses of particles do not given a corresponding increase in liver copper stores perhaps confirm this (MacPherson, 1984; Suttle, 1987).

A single dose of 4 g for calves and 20 g for beef cows, which provided 3.2 g and 16 g copper respectively, protected initially deficient animals throughout the grazing season (MacPherson, 1983). The use of 20 g copper oxide needles in growing cattle has been shown to increase liveweight gain and prevent the severe copper deficiency seen in unsupplemented animals (Whitelaw, Fawcett & MacDonald, 1984).

The increases in blood plasma copper concentrations and liver copper concentrations recorded for the administration of copper oxide needles has been shown to be greater than or equal to that recorded in similar calves given parenteral copper supplementation (Richards et al., 1985; Rogers & Poole, 1988). The recommendations for dose rates of copper oxide needles are: calves (under 100 kg) 4 g; growing cattle (100-300 kg) 24 g and adult cattle (over 300 kg) 24-48 g.

This method allows large amounts of copper to be administered in a single dose, sufficient to last the grazing season.

**Soluble glass bolus**

This is composed of a phosphate based glass into whose structure copper oxide, elemental selenium and cobalt oxide are incorporated (Telfer, Zervas & Knott, 1983). The resultant glass is moulded into a cylindrical bolus, two of which should be administered to the animal by means of an oesophageal balling gun. The bolus has a density of 2.8 $g/cm^3$ and is designed to be retained within the reticulo-rumen and to dissolve at a controlled rate.

The rate of release of material from the bolus is a function of surface area (Knott et al., 1985) and therefore decreases with time. Six months after administration the release rate will have fallen to less than 50% of the initial release rate (Zervas et al., 1988).

The copper and selenium supplied by the soluble glass bolus have been shown to increase blood plasma and liver copper concentrations and blood glutathione peroxidase activities in both growing and adult cattle for up to 300 days (Telfer, Zervas & Carlos, 1984; Allen et al., 1984; Judson, Brown & Dewey, 1985; Knott et al., 1985; Judson et al., 1985; Buckley, Strachan & Puls, 1987; Givens et al., 1988). The selenium status of calves born to bolused dams has been shown to be improved and milk selenium levels increased by giving soluble glass boluses (Hidirigolou & Proulx, 1988).

There is very little evidence however of the effectiveness of the cobalt supplied by the soluble glass boluses in increasing plasma Vitamin $B_{12}$ concentrations and often no response is observed (Allen, Drake & Tripp, 1985).

The soluble glass bolus was withdrawn in 1986 due to problems with surface deterioration caused by moisture permeating through the packaging. This surface deterioration may have led to increased susceptibility to fragmentation of the bolus within the rumen (Judson et al., 1988). This problem of fragmentation of the soluble glass bolus was noted in trials with a version of the bolus suitable for administration to sheep (Judson et al., 1985; Millar et al., 1988).

A new formulation of the soluble glass bolus has been developed and was marketed in 1990. It used a sintered glass containing copper oxide, sodium selenate and cobalt oxide. Each bolus weighs 100 g and again two boluses are the recommended dose. Presumably to overcome the problems of surface deterioration and fragmentation the boluses are covered in a papier-mache sheath and then sealed in a foil packaging.

# SECTION 1

## 1.1

## Background Information

The rumen bolus described in this thesis is a patented invention of the University of Glasgow (Hemingway, Ritchie & Parkins, 1986). The bolus is designed to be completely degradable and to leave no residue.

Its basis is a compressed mixture of mineral salts in cylindrical form (about 8.0 cm length x 2.5 cm diameter) which is coated with a polymer resin to leave one exposed end. The resin coating progressively breaks off to maintain a constant surface area which should allow a linear release rate.

A treatment consists of two boluses. These are given orally by the use of an oesophageal balling gun which delivers the bolus to the back of the tongue, from which point they are swallowed by the animal. Two boluses are administered as the release of material is partly by dissolution and partly by mutual erosion. The density of the boluses is such that they are not regurgitated and are retained in the reticulo-rumen. Initial development of the prototype was reported by Simpson (1985). In that work the aim was to supply the total requirement of a 150 kg animal with six trace elements: copper, cobalt, selenium, manganese, zinc, iodine and smaller quantities of Vitamin A/$D_3$ and Vitamin E over a period of 100 days.

The bolus was formulated to supply these from readily available mineral salts most of which were already used in commercial trace element mixes. The basis for the initial formulation is shown in Table 1.1. A pair of boluses were to be given and each bolus was to weigh 50 g and have an expected life of 100 days, i.e. a release rate of 0.5 g/bolus/day to supply a total of 1.0 g material/day.

Table 1.1 shows that such a bolus would supply the total requirement for a 150 kg animal of all 6 trace elements and Vitamins A and D and would supply a useful quantity of Vitamin E.

The initial development work stopped in 1985 but after that date Dr N S Ritchie continued and made two major changes in altering the shape of the bolus and in changing the type of resin used for the coating. Both of these were to allow dip coating of the boluses. This method gave superior coating to the brush method employed by Simpson (1985a) and that relatively poor coating may have caused many of the irregular results reported in that work.

This thesis describes the development work with the bolus starting from October 1986 with the aim of providing a substantial proportion of the trace element requirements of all sizes of cattle above 150 kg for up to one year.

**Table 1.1 Initial composition of the trace element bolus (Simpson, 1985) and comparison of the expected supply with requirements of a 150 kg animal consuming 5 kg DM/day.**

| Component | g/100 g | % nutrient | Daily release from two boluses mg | Requirement mg/day | **% supplied by two boluses** |
|---|---|---|---|---|---|
| Copper oxide needles | 25.0 | 84 | 210 | 60 | **350** |
| Manganese sulphate | 35.8 | 31 | 111 | + 100 | **111** |
| Zinc sulphate heptahydrate | 12.5 | 22 | 172 | + 110 | **156** |
| Zinc oxide | 18.1 | 80 | | | |
| Cobalt sulphate | 0.27 | 21 | 0.6 | 0.55 | **109** |
| Sodium selenite | 0.13 | 45 | 0.6 | 0.50 | **120** |
| Potassium iodate | 0.49 | 59 | 2.9 | ** 0.75 | **387** |
| Vitamin A/$D_3$ | 3.88 | * | 19,400 iu | 16,500 iu | **118** |
| | | * | 3880 iu | 1500 iu | **259** |
| Vitamin E | 3.88 | 50 | 19.4 | 75 | **26** |

* Vitamin A/$D_3$ contains 500,000 iu A, 100,000 iu $D_3$ per g.

\+ These requirements are from ARC (1980), the allowances in MAFF et al. (1983) are substantially greater for manganese and zinc.

** In the presence of goitrogens the requirement increases to 10 mg/day.

## 1.2
## Laboratory production of the bolus
## Materials and methods

The materials used in the preparation of the experimental boluses are listed below. Any mixtures of these substances in powder form and when compressed to form a bolus subsequent to coating were termed the bolus 'matrix'.

Matrix components:

| | |
|---|---|
| Copper oxide powder | CuO |
| Manganese sulphate monohydrate | $MnSO_4H_2O$ |
| Zinc oxide | ZnO |
| Zinc sulphate heptahydrate | $ZnSO_47H_2O$ |
| Potassium iodide | KI |
| Cobalt sulphate | $CoSO_47H_2O$ |
| Sodium selenite | $Na_2SeO_3$ |
| Vitamin A/D3 | |
| Vitamin E | |

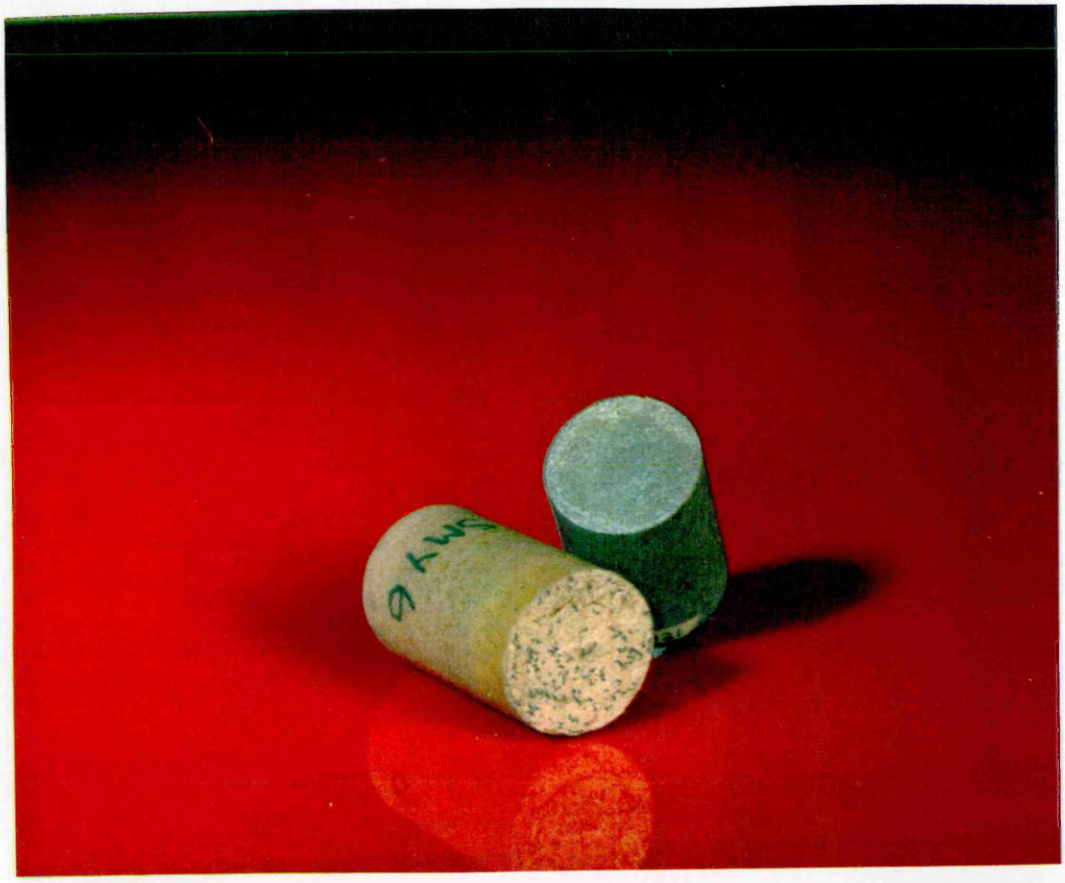
Full specifications of these materials are given in Appendix 1. All the materials were passed through a 600 um sieve before use, but the zinc sulphate heptahydrate required to be passed through a mechanical grinder as a preliminary step.

Copper oxide powder was used in preference to the original copper source, copper oxide 'needles', as it mixed better (See Plate 3). Copper oxide powder as well as being the source of copper was also the major weighting agent.

The materials were weighed out in the required amounts into individual containers and once all were weighed out they were poured into a polythene container and thoroughly mixed by gentle agitation. The process was completed by placing the mix into a mechanical mixer for 10-15 seconds.

The material could then be weighed out into individual containers for pouring into the die to form a bolus.

**Plate 3.**
**Copper oxide powder was used in preference to copper oxide needles to allow better mixing.**



## 1.3

## Press Technology

The press used for this work was a small manually operated hydraulic bench press (Tangye Hydraulics Ltd: Type PRE 80B) with a 10 tonne ram. In March 1988 an electric pump (HES Ltd., Hiforce 20000 Series) was fitted which allowed easier, faster and more efficient bolus manufacture.

The dies and plungers to fit this press were manufactured by the Mechanical Engineering Department of Glasgow University. The dies were 130 mm in length and were bored out in the centre to two sizes, 25 mm and 19 mm in diameter. A base plate with an insert of the correct diameter was placed at the bottom of the die during filling and compression and could be removed to allow the bolus to be expelled. The insert was machined out so that the base of the bolus was curved as a hemisphere.

This equipment allowed the compression of boluses up to 7.5 $t/in^2$, this pressure being applied to the top surface of the bolus.

## 1.4

## Coating of the boluses

The compressed boluses are coated such that the flat end surface is the only exposed area. This means that almost linear release of material can be achieved in the rumen since there is a constant surface area subject to dissolution and erosion.

The material used was a preaccelerated isopthalic polyester resin (Crystic 491PA) catalysed by MEKP (Scott Bader Ltd). This resin has a low level of residual styrene and is thus non-toxic and non-tainting. As such it has been approved for use as containers for food and potable liquids.

The resin can be applied by brush or spray but for the bolus a dip coating was preferred. The compressed boluses had a string attached to their flat top surface with instant glue. They could then be suspended from a horizontal rod and a beaker of catalysed resin raised around the bolus until the whole bolus was submerged. All stages of the coating process were carried out in a fume cupboard.

After the initial resin coating had dried a second coat was applied as the standard technique to ensure that there were no flaws in the surface where rumen liquid could penetrate and cause the bolus to erode from that point.

The resin formulation used for coating was Crystic 491PA (100 parts) and Catalyst MEKP (2 parts). With laboratory temperatures of around 20$^{o}$C the setting time for this resin is 18 minutes. The interval between coats was a minimum of 4 hours. The resin was then allowed to cure at laboratory temperature for a minimum of 24 hours.

The resin coating over the top surface to which the string was fastened was easily removed leaving a flat exposed end. For most of the experimental work the boluses were then tested in rumen fistulated cows within a few days of manufacture. If the boluses were to be stored exposed to the atmosphere for more than a few days the exposed end was dipped in a low temperature melting point paraffin wax to prevent deterioration as a result of dampness.

## 1.5

## Standard materials and methods for bolus production and testing and results for bolus Matrix No 1.

With the main restraint on this work being the number of fistulated cows available it was important that the materials and methods were standardised to allow direct comparison between different bolus formulations. This section outlines these to avoid unnecessary repetition and unless otherwise stated throughout this thesis the materials and methods were as follows.

### Bolus composition

The initial formulation for the bolus was chosen for reasons outlined in Section 1.1. From this the standard mix was:

| | g/100 g |
|---|---|
| Manganese sulphate monohydrate | **47.68** |
| Zinc sulphate heptahydrate | **16.67** |
| Zinc oxide | **24.13** |
| Cobalt sulphate | **0.36** |
| Sodium selenite | **0.17** |
| Potassium iodide | **0.65** |
| Vitamin $A/D_3$ | **5.17** |
| Vitamin E | **5.17** |

In the initial work (Simpson 1985b) this standard mix was added to copper oxide needles in the ratio 3 to 1 (75 to 25) to form the bolus matrix. However copper oxide in the form of needles was found not to mix well with other ingredients in a powdered form. This was shown to be particularly so during an automated bolus-pressing process where machine vibration caused separation of the dense copper oxide needles. This present work thus used copper oxide in the form of a powder in order to obtain a uniform mixture with other powder form ingredients. Simpson (1985c) found that the bolus release rate using copper oxide powder was negligible over 51 days whereas with copper oxide needles a mean release rate of 0.331 g/bolus/day was recorded over that period.

In anticipation of this problem an additional 5% zinc sulphate heptahydrate, which is the most soluble component of the mix, was used to form Matrix No 1.

**Bolus formulation g/100 g**

| Component | g/100 g |
|---|---|
| standard mix | 70 |
| Copper oxide powder | 25 |
| Zinc sulphate heptahydrate | 5 |

**Bolus manufacture**

Preparation and mixing of materials was as detailed in Section 1.2 with a bolus weight of 100 g. This was compressed at 7.5 t/in$^2$ for 10-15 seconds.

**Bolus coating**

Materials and procedures were as in 1.4 with no pigment being added and two coats of resin were applied by dipping.

**Measurement of bolus release rate**

Two boluses were placed in the reticulum of each of three rumen fistulated cows and were removed at weekly intervals at 10.00 hours.

When removed the boluses were washed, damp dried with paper towels and weighed. The loss in weight over the week was calculated and divided to give the daily release over that 7 day period.

Two groups each of three fistulated cows were used in this experiment. One group were given hay and 2 kg concentrates to day 56 and were then transferred to grass and the boluses evaluated to day 119. The second group were at grass and the boluses were tested to day 56.

**Results**

The results for Matrix No 1 are shown in Table 1.2 and Figure 1.1 shows the results from Group 1.

In this and in subsequent tables s.d. refers to the standard deviation of the release rates of the individual boluses rather than the pairs within cows. SED refers to the standard error of the difference between means.

Inclusion of additional zinc sulphate heptahydrate appears to overcome the problem of low weight loss encountered with boluses containing copper oxide powder and release rates are only 20% lower than those reported for boluses containing copper oxide needles (Simpson, 1985).

The results for the three cows in Group 1 indicate that when the high initial release rates in days 0-14 or 0-21 are excluded the boluses lose weight at about the same rate when given hay and concentrates or when at grass.

After the period 0-7 days the mean daily release rate for the three cows in Group 2 were very similar to those in Group 1. This suggests that evaluation of bolus types in pairs in each of three cows is an adequate initial test. Plate 4 shows a pair of boluses composed of Matrix No 1 and Plate 5 shows similar boluses after testing in fistulated cows.

It is estimated that boluses of such a composition and weighing 100 g would have a life of about 400 days, this gives an estimated daily provision from two boluses of 97 mg copper, 0.25 mg cobalt, 0.25 mg selenium, 1.55 mg iodine, 52 mg manganese, 87 mg zinc, 9000 iu Vit A, 1800 iu $D_3$ and 9 mg Vit E.

**Table 1.2** **Mean bolus release rate (g/bolus/day) for bolus Matrix No 1 (initial weight 101 g).**

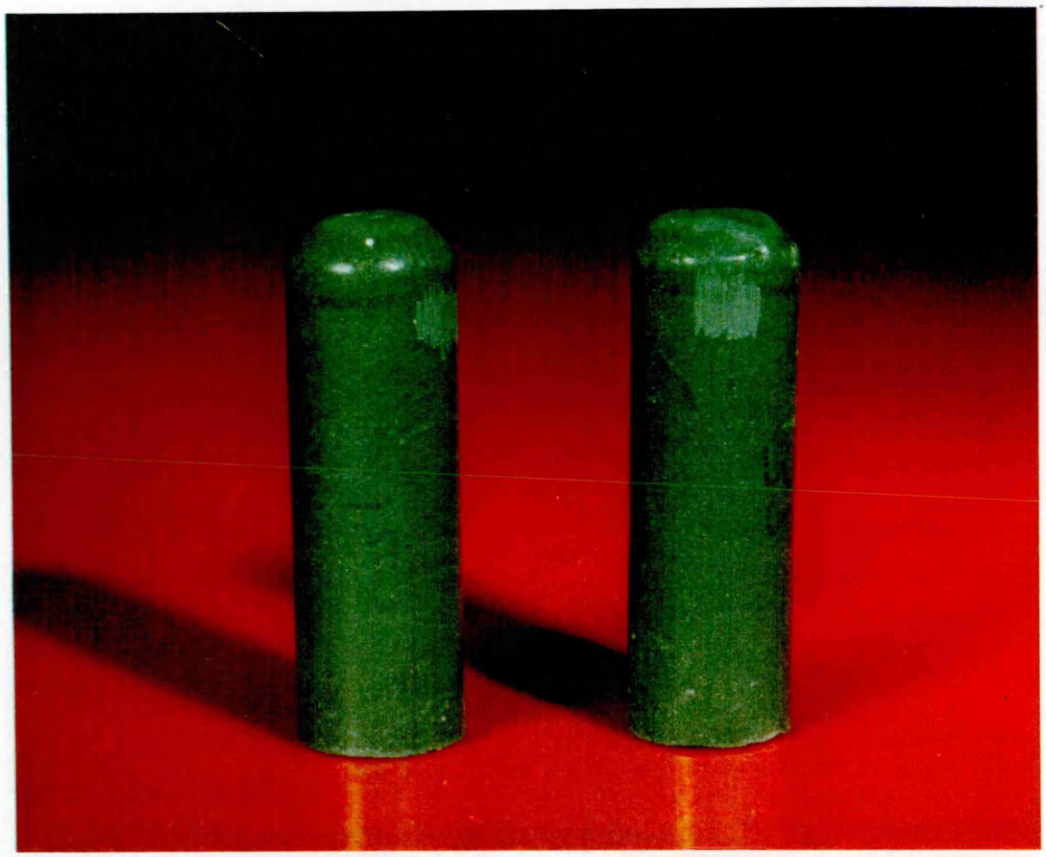
| | Group 1 Hay and concentrates | | Group 2 At grass | |
|---|---|---|---|---|
| **Days** | mean | s.d. | mean | s.d. |
| **0-7** | 0.37 | 0.122 | 0.47 | 0.105 |
| **8-14** | 0.35 | 0.125 | 0.27 | 0.049 |
| **15-21** | 0.31 | 0.076 | 0.24 | 0.051 |
| **22-28** | 0.22 | 0.032 | 0.23 | 0.032 |
| **29-35** | 0.21 | 0.044 | 0.21 | 0.047 |
| **36-42** | 0.26 | 0.020 | 0.22 | 0.027 |
| **43-49** | 0.18 | 0.059 | 0.15 | 0.071 |
| **50-56** | 0.24 | 0.061 | 0.21 | 0.071 |
| **mean 0-56** | **0.27** | 0.030 | **0.25** | 0.030 |
| **mean bolus wt at day 56** | **85.8** | 1.55 | **87.0** | 1.59 |

**mean loss 0-56 days** **Hay/conc vs Grass** **SED 0.014 (NS)**

| | At grass | |
|---|---|---|
| **57-63** | 0.17 | 0.044 |
| **64-70** | 0.19 | 0.044 |
| **71-77** | 0.17 | 0.014 |
| **78-84** | 0.20 | 0.010 |
| **85-91** | 0.26 | 0.005 |
| **92-98** | 0.19 | 0.039 |
| **99-105** | 0.24 | 0.037 |
| **106-112** | 0.26 | 0.037 |
| **113-119** | 0.26 | 0.039 |
| **mean 57-119** | **0.22** | 0.025 |
| **mean 0-119** | **0.24** | 0.024 |
| **mean bolus wt at day 119** | **72.1** | 2.95 |

**Plate 4.**
**A pair of boluses composed of Matrix No 1.**

**Plate 5.**
**A single bolus of Matrix No 1 from pairs removed from testing in fistulated cows after 42, 84 and 119 days respectively.**



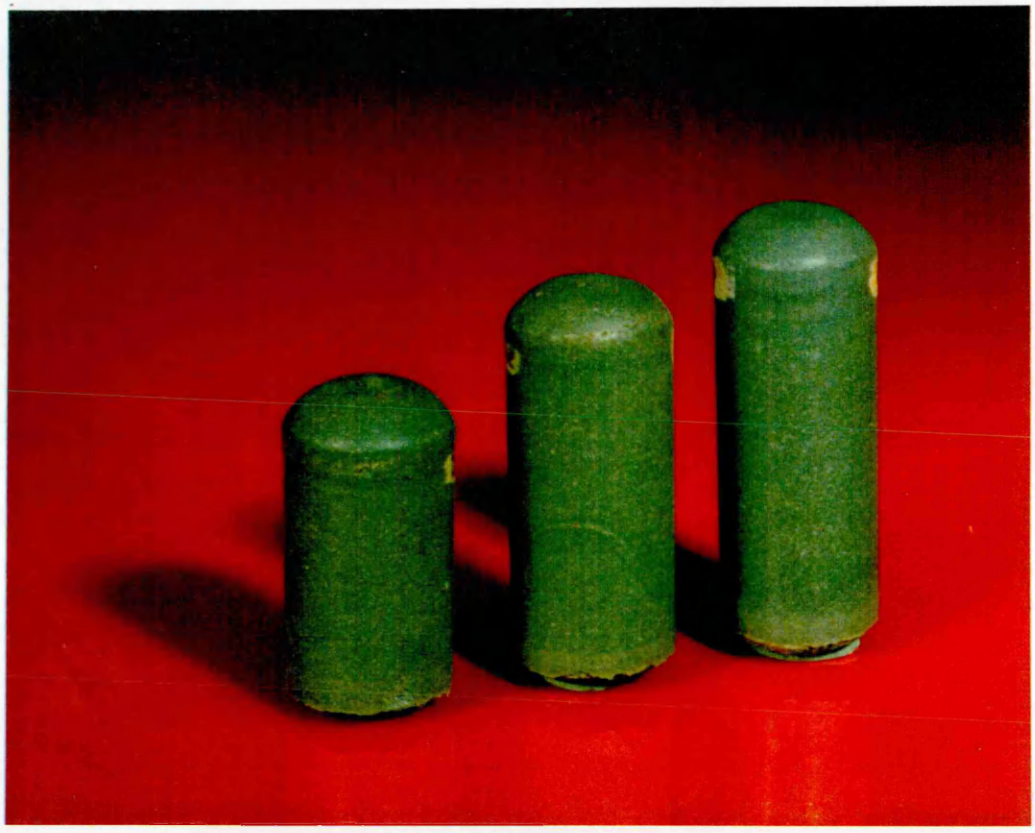
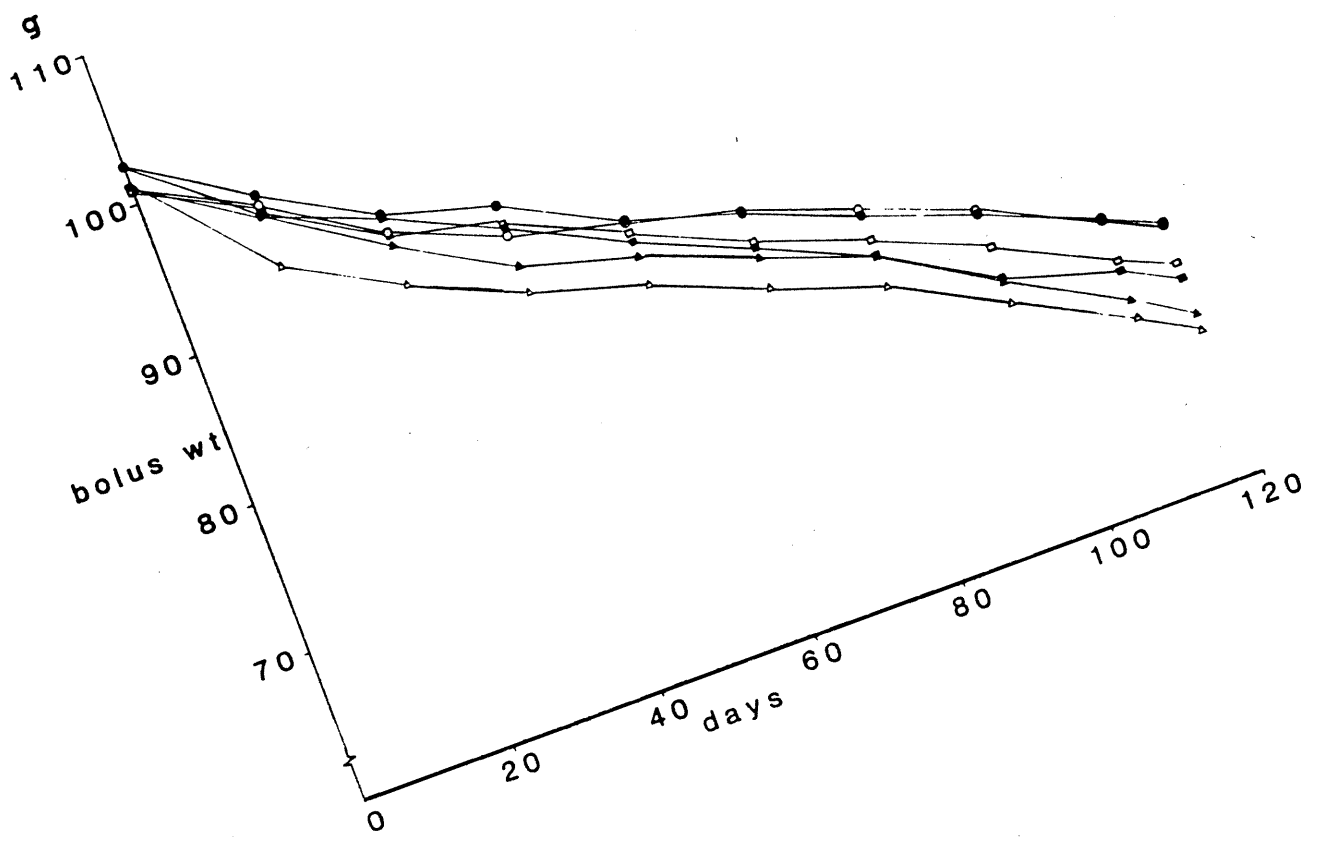


**Figure 1.1**
**The mean loss in weight of six boluses of Matrix No 1 tested in fistulated cows fed hay and concentrates to day 56 and thereafter at grass.**


g
110
100
90
bolus wt
80
70
0
20
40
days
60
80
100
120

## SECTION 2

**The development of the multiple trace element bolus.**

This section describes a series of experiments which examine the factors affecting the release rate of the multiple trace element bolus. These factors include:

Bolus matrix formulation
Bolus weight and coating
Number of boluses administered
Presence of other bolus products and their residues

Finally this section describes the development and testing of the commercially manufactured bolus and gives an assessment of the adequacy of the trace elements supplied by it.

# SECTION 2

**Experiment 2.1**
**The effect on bolus release rate (g/bolus/day) of inclusion within the bolus matrix of different grades of zinc oxide.**

**Experiment 2.2**
**The effect on bolus release rate (g/bolus/day) of increasing the inclusion of zinc sulphate heptahydrate within the bolus matrix.**

**Experiment 2.3**
**The effect on bolus release rate (g/bolus/day) of increasing the inclusion within the bolus matrix of copper oxide powder.**

**Experiment 2.4.**
**The effect on bolus release rate (g/bolus/day) of different Vitamin A/$D_3$ formulations and their level of inclusion.**

**Experiment 2.5.**
**The effect on bolus release rate (g/bolus/day) of increasing the inclusion of cobalt and selenium within the bolus matrix.**

**Experiment 2.6.**
**The effect on bolus release rate (g/bolus/day) of reducing the bolus weight.**

**Experiment 2.7.**
**The effect on bolus release rate (g/bolus/day) of the number of resin coats applied to the bolus.**

**Experiment 2.8.**
**The effect on bolus release rate (g/bolus/day) of the number of boluses administered.**

**Experiment 2.9.**
**The effect on bolus release rate (g/bolus/day) of the presence of other boluses and their residues within the reticulum.**

**Experiment 2.10.**
**A survey of the number and type of bolus residues found in cattle at slaughter.**

**Development of the mutliple trace element bolus as a commercial product.**

**Experiment 2.11.**
**Evaluation of the 'All-trace' bolus in rumen-fistulated cows.**

**Assessment of the trace element contribution from the 'All-trace' bolus to the nutrient intake of cattle grazing herbage of inadequate trace element content.**

**Experiment 2.12**
**An assessment of the uniformity of individual composition of boluses within a particular production batch both before and after administration to cattle.**

## Experiment 2.1

### The effect on bolus release rate (g/bolus/day) of inclusion within the bolus matrix of different grades of zinc oxide.

### Introduction

Zinc oxide is typically a grey white powder virtually insoluble in water. It compresses well even with hand pressure.

The material which had been used in the preliminary bolus work by Simpson (1985) was a product 'Vidox' which was described as 98% zinc oxide. This was widely used as an animal feed grade material because it was of lower purity than other products and was consequently less expensive.

'Vidox' became unavailable during 1986 and an alternative material 'Vitazinc' was investigated. This product was a fine brown powder containing approximately 93% zinc oxide.

Since the purity of 'Vitazinc' was so much lower than 'Vidox' it was decided to test a further alternative product 'Silverseal' which contained 99.5% ZnO.

This experiment examines the effect of inclusion of Vitazinc and Silverseal zinc oxides as compared to Vidox.

### Materials and methods

These were as in Section 1.5 with 'Vitazinc' or 'Silverseal' replacing Vidox as the zinc oxide component within Matrix No 1. The test period was 21 days and the three cows in each group were given hay and 2 kg concentrates for the whole of that period.

### Results

These are shown in Table 2.1. Those boluses containing Vitazinc were removed from testing after 7 days since the residual bolus weight at that point ranged from only 3 to 12 g. All the boluses were recovered and those with 'Vidox' or 'Silverseal' showed even patterns of wear at the exposed end surfaces.

The inclusion of 'Vitazinc' ZnO in the bolus matrix caused the bolus release rate to increase to 27 times that of the original 'Vidox' product. Additional observations with a single cow suggested that the release of material occurred

**Table 2.1. The effect on bolus release rate (g/bolus/day) of the inclusion of three different types of zinc oxide.**

| | Zinc Oxide Type | | | | | |
|---|---|---|---|---|---|---|
| | 'Vitazinc' | | 'Vidox' | | 'Silverseal' | |
| Release rate | mean | s.d. | mean | s.d. | mean | s.d. |
| **Days** | | | | | | |
| **0-7** | 13.4 | 0.49 | 0.50 | 0.081 | 0.44 | 0.206 |
| **8-14** | - | - | 0.35 | 0.057 | 0.26 | 0.069 |
| **15-21** | - | - | 0.37 | 0.059 | 0.30 | 0.160 |
| **mean** | | | | | | |
| **0-7** | **13.45*** | 0.49* | | | | |
| **0-21** | | | | | | 0.107 |
| | | | **0.41** | 0.048 | **0.34** | |
| **mean final** | | | | | | |
| **bolus wt. (g)** | **6.9** | 3.42 | **91.4** | 1.02 | **92.9** | 2.26 |

* Boluses removed on day 7.
Mean loss 0-21 days Vidox v Silverseal SED 0.068 NS.

almost immediately (only 11 g and 9 g of two boluses originally weighing 101 and 102 g.remained after 4 days). This was obviously unacceptable.

'Silverseal' ZnO showed no significant difference in overall mean release rate to that of Vidox over the 21 day period (0.34 and 0.41 g/day). It was therefore adopted as the zinc oxide source and was used in all subsequent bolus matrices reported in this thesis.

All three zinc oxides were analysed for silica to assess if this impurity in Vitazinc was the reason for the drastic effect on bolus release rate. The results were 7.5, 8.2 and 62.6 g/kg for Vidox, Silverseal and Vitazinc respectively. It may be that the high level of silica in Vitazinc caused the bolus matrix to disintegrate when exposed to the rumen environment.

**Experiment 2.2**

**The effect on bolus release rate (g/bolus/day) of increasing the inclusion of zinc sulphate heptahydrate within the bolus matrix.**

**Introduction**

The most soluble major component of the bolus matrix was zinc sulphate heptahydrate (Zn $SO_4$ $7H_20$) which will dissolve in less that its own weight of water. It was considered that one method of increasing the overall release rate of the bolus would be to increase the content of zinc sulphate heptahydrate.

This experiment compares the higher levels of zinc sulphate $7H_20$ (10 and 15%) with the 5% level as used in Matrix No 1 the release rate of which has already been described in Table 1.2.

**Materials and methods**

These were as in Section 1.5 except that the bolus formulations were:

| Component | g/100 g | | |
|---|---|---|---|
| Zinc sulphate $7H_20$ | **5** | **10** | **15** |
| Copper oxide powder | **25** | **25** | **25** |
| Standard mix | **70** | **65** | **60** |

The test period was 56 days and the cows were at grass for the whole of that period.

**Results**

These are given in Table 2.2. The results for the 5% inclusion of Zinc sulphate heptahydrate taken from Table 1.2 are shown here for comparison.

Increasing the level of zinc sulphate from 5 to 10% and then to 15% accelerated the bolus release rate from 0.25 to 0.30 and to 0.55 g/day respectively. The results were as expected but the variation in release rates between boluses of the same type was also greater as the zinc sulphate inclusion increased. The coefficients of variation for mean daily release rate for 5, 10 and 15% inclusions were 13, 31 and 38% respectively. The high rates of release may be one reason for this but another important factor could be the problem of thoroughly mixing a bolus matrix with 15% added zinc sulphate heptahydrate. The full specification of the material is given in Appendix 1, but briefly it is a crystalline material of large particle size, almost entirely larger than 600 um. With a great deal of processing it is possible to allow it to pass through a 600 um sieve. In contrast, 100% of copper oxide powder will pass through a 75 um sieve.

Zinc sulphate heptahydrate is therefore a very soluble component of the bolus matrix and increasing the level of inclusion will increase bolus release rate. However, the physical properties of the material make it difficult to mix evenly and this may account for the increased variation in bolus release rate observed in this experiment.

**Table 2.2. The effect on bolus release rate (g/bolus/day) of increasing the inclusion rate of zinc sulphate heptahydrate within the bolus matrix.**

| | % $ZnSO_4$ $7H_2O$ | | | | | |
|---|---|---|---|---|---|---|
| | 5* | | 10 | | 15 | |
| **Days** | mean | s.d. | mean | s.d. | mean | s.d |
| **0-7** | 0.47 | 0.105 | 0.53 | 0.234 | 0.74 | 0.306 |
| **8-14** | 0.27 | 0.049 | 0.35 | 0.169 | 0.46 | 0.171 |
| **15-21** | 0.24 | 0.051 | 0.26 | 0.076 | 0.35 | 0.064 |
| **22-28** | 0.23 | 0.032 | 0.33 | 0.083 | 0.33 | 0.076 |
| **29-35** | 0.21 | 0.047 | 0.25 | 0.061 | 0.40 | 0.157 |
| **36-42** | 0.22 | 0.027 | 0.24 | 0.093 | 0.72 | 0.443 |
| **43-49** | 0.15 | 0.071 | 0.21 | 0.076 | 0.74 | 0.463 |
| **50-56** | 0.21 | 0.071 | 0.24 | 0.064 | 0.54 | 0.208 |
| **mean 0-56** | **0.25** | 0.030 | **0.30** | 0.093 | **0.55** | 0.208 |
| **mean final bolus wt. g** | **87.0** | 1.59 | **83.5** | 5.16 | **70.6** | 11.60 |

* from Table 1.2 Group 2.

**Mean loss 0-21 days** 10% v 15% SED 0.080 $P<0.01$

**Experiment 2.3.**

**The effect on bolus release rate (g/bolus/day) of increasing the inclusion within the bolus matrix of copper oxide powder.**

**Introduction**

The copper oxide in the bolus formulation has two functions. It is the source of copper and it is also the major weighting agent used to increase the bolus density. The inclusion was originally set as 25% since this level was calculated to give the bolus a density of 2.5-2.7 $g/cm^3$. This experiment examines the effect of increasing the proportion of copper oxide from 25% to 35% in an attempt to increase the bolus density.

**Materials and methods**

These were as in Section 1.5. except that the bolus formulation was:

| Component | g/100 g |
|---|---|
| Copper oxide powder | 35 |
| Standard mix | 60 |
| Zinc sulphate. $7H_2O$ | 5 |

The test period was 56 days and the cows were given hay and 2 kg concentrates for the whole of that period.

**Results**

The density of the bolus was 2.65 g/cm$^3$ compared to 2.6 g/cm$^3$ for the standard 25% copper oxide powder inclusion. The mean daily release rate is compared with that for 25% copper oxide inclusion from Table 1.1. and shown in Table 2.3.

The increased inclusion of copper oxide did not increase the bolus density by an appreciable amount. It did however reduce the release rate significantly ($P < 0.001$). This result indicates that the density of the trace element bolus could not be increased significantly by increasing the proportion of copper oxide powder.

**Table 2.3. The effect on bolus release rate (g/bolus/day) of increasing the inclusion of copper oxide powder within the bolus matrix.**

| | % Copper oxide | | | |
|---|---|---|---|---|
| | 25* | | 35 | |
| **Days** | mean | s.d. | mean | s.d. |
| **0-7** | 0.37 | 0.122 | 0.27 | 0.056 |
| **8-14** | 0.35 | 0.125 | 0.15 | 0.026 |
| **15-21** | 0.31 | 0.076 | 0.16 | 0.018 |
| **22-28** | 0.22 | 0.032 | 0.16 | 0.021 |
| **29-35** | 0.21 | 0.044 | 0.07 | 0.015 |
| **36-42** | 0.26 | 0.020 | 0.10 | 0.010 |
| **42-49** | 0.18 | 0.059 | 0.11 | 0.006 |
| **50-56** | 0.24 | 0.061 | 0.10 | 0.010 |
| **mean 0-56** | **0.27** | 0.030 | **0.14** | 0.014 |
| **mean final bolus wt. (g)** | **85.8** | 1.55 | **93.5** | 1.93 |

* from Table 1.1, Group 1.

Mean loss 0-21 days SED 0.012 $P < 0.001$

**Experiment 2.4.**

**The effect on bolus release rate (g/bolus/day) of different Vitamin A/$D_3$ formulations and their level of inclusion.**

**Introduction**

The Vitamin A/$D_3$ used in the bolus formulation was Rovimix A/$D_3$ Type 500:100 (Roche Products Ltd). It contained 500,000 iu Vitamin A and 100,000 iu Vitamin $D_3$ per gramme. The preparation is a free flowing brown powder with 100,000 particles/g, within these the vitamins are stablised in a matrix of gelatin, glycerine and carbohydrate.

Until August 1986 Rovimix A/$D_3$ was available as Type M. This was then replaced with Type P. This material was different in that the grade of gelatin and the pre-heat treatment had changed.. The change was made to reduce losses of Vitamin A during the longer conditioning times and higher pelleting temperatures being used in animal feed manufacture. Vitamin A/$D_3$ accounts for only 3.62% of the Matrix No. 1 bolus formulation but initial trials suggested that the changes made to this material could have major effects on the bolus release rate.

This experiment examines the effect of the change from Type M to Type P Vitamin A/$D_3$ on bolus release rate and evaluates the effect of different rates of inclusion of Type P A/$D_3$.

**Materials and Methods**

These were as in Section 1.5. with five formulations being evaluated as pairs of boluses in each of three fistulated cows. The formulations were all based on Matrix No 1. The boluses weighed 100 g and the various Vitamin A/$D_3$ inclusions were:

| | |
|---|---|
| A | No Vitamin A/$D_3$ |
| B | Type P 25% of Matrix No 1 inclusion |
| C | Type P 50% of Matrix No 1 inclusion |
| D | Type P 100% of Matrix No 1 inclusion |
| E | Type M 100% of Matrix No 1 inclusion, i.e as for original |

Where the inclusion was less than 100% of the original Matrix No 1 inclusion (i.e. 3.62% of the total bolus matrix) the difference was allowed for by small additional amounts of all the ingredients except copper oxide.

The test period was 35 days and the cows were given hay and 2 kg concentrates for the whole of that period.

**Results**

These are presented in Table 2.4.

When Type P Vitamin A/D3 formulation was substituted at the same rate of inclusion for Type M (as originally used in Bolus Matrix No 1) the overall mean loss in weight per bolus per day was greatly increased (2.17 g (D) v 0.31 g (E). As the inclusion of Type P was decreased to 50% of the original (C) and then to 25% (B) and 0% (A) the daily release rate fell progressively to 1.64, 0.74 and 0.22 respectively.

Within each of the formulations A - E evaluated the coefficient of variation for each mean overall daily release rate was in the order of 5-8% and accordingly the boluses of each type lost weight in a comparatively uniform manner.

It is disturbing to record such marked changes in mean bolus weight loss for such small alterations in bolus composition. It is therefore imperative that both the type of Vitamin A/$D_3$ formulation and its rate of inclusion and mixing must be closely controlled if a uniform and predictable bolus release rate is to be achieved.

**Table 2.4. The mean effects on bolus release rates (g/bolus/day) of different Vitamin A/D3 formulations and their level of inclusion relative to Bolus Matrix No 1 (Type M).**

| | A Type P | | B Type P | | C Type P | | D Type P | | E Type M | |
|---|---|---|---|---|---|---|---|---|---|---|
| % of Matrix No 1 inclusion | 0% | | 25% | | 50% | | 100% | | 100% | |
| **Day** | mean | s.d. | mean | s.d. | mean | s.d. | mean | s.d. | mean | s.d. |
| **0-7** | 0.27 | 0.025 | 0.81 | 0.036 | 2.89 | 0.365 | 4.90 | 0.251 | 0.43 | 0.056 |
| **8-14** | 0.22 | 0.017 | 0.77 | 0.067 | 2.02 | 0.117 | 1.47 | 0.186 | 0.29 | 0.055 |
| **15-21** | 0.18 | 0.020 | 0.75 | 0.058 | 1.32 | 0.257 | 1.60 | 0.288 | 0.27 | 0.063 |
| **22-28** | 0.22 | 0.021 | 0.84 | 0.150 | 1.08 | 0.134 | 1.53 | 0.267 | 0.28 | 0.046 |
| **29-35** | 0.23 | 0.034 | 0.54 | 0.077 | 0.90 | 0.039 | 1.34 | 0.103 | 0.29 | 0.047 |
| **Overall mean 0-35** | **0.22** | 0.013 | **0.74** | 0.058 | **1.64** | 0.116 | **2.17** | 0.107 | **0.31** | 0.026 |
| **Mean final wt. (g)** | **93.2** | 0.40 | **75.2** | 2.05 | **43.5** | 4.04 | **25.1** | 3.74 | **90.2** | 0.89 |

**Mean loss 0-21 days** A<E; B,C,D>E P<0.001

**Experiment 2.5.**

**The effect on bolus release rate (g/bolus/day) of increasing the inclusion of cobalt and selenium within the bolus matrix.**

**Introduction**

The results from Section 1.5 indicated that a mean weight loss of 0.25 g of bolus matrix per day, i.e. 0.5 g from two boluses could be expected from Matrix No 1. This would provide the following amounts of cobalt and selenium:

| | g/100 g matrix | Nutrient content% | mg provided by 0.5 g bolus matrix |
|---|---|---|---|
| Cobalt sulphate | **0.25** | **21** | **0.25** |
| Sodium selenite | **0.12** | **45** | **0.25** |

The report of a Joint Working Party composed of the Ministry of Agriculture, Fisheries and Food, Department of Agriculture for Scotland, Department of Agriculture for Northern Ireland, United Kingdom Agricultural Supply Trade and the British Veterinary Association (MAFF et al, 1983) have concluded that dietary allowances (which include safety margins) should be 0.11 mg/kg DM intake for cobalt and 0.10 mg/kg DM intake for selenium. If it is assumed that 150 and 500 kg cattle consume 5 and 10 kg DM respectively their total dietary allowances for cobalt would be 0.55 and 1.1 mg respectively and 0.5 and 1.0 mg for selenium.

The above contributions from two boluses would provide about half the total allowance of each for 150 kg cattle and about 25% for 500 kg cattle. It should however be borne in mind that inadequate herbage never contains nil cobalt and selenium. Perhaps 0.04 mg Co and 0.03 mg Se/kg DM respectively would be the lowest concentrations to be encountered under field conditions (MacPherson, A. 1988; Hemingway, R.G. 1988; Mills, C.F. 1988; Personal Communications). The amounts required from a bolus would be those to supplement these quantities.

This experiment investigates the effects of including increased amounts of cobalt and selenium in the bolus matrix.

## Materials and Methods

These were as in Section 1.5. but with two formulations containing increased amounts of cobalt sulphate and sodium selenite.

| | Original inclusion | Increased inclusions | |
|---|---|---|---|
| | | g/100 g matrix | |
| | | A | B |
| Cobalt sulphate | **0.25** | **0.50** | **1.00** |
| Sodium selenite | **0.12** | **0.24** | **0.48** |

Matrixes A and B were constructed to contain two and four times the quantity used in Matrix No 1. The quantities of the other matrix components were adjusted accordingly. The mean initial bolus weight was 100 g. The test period was 42 days and the cows were fed hay and 2 kg concentrates for the whole of that period.

## Results

Increasing the concentrations of cobalt sulphate and sodium selenite included in the bolus matrix to twice that in Matrix No 1 had no significant effect on the weight loss of each bolus which was about 0.31 g per day over the 42 day period (Table 2.5.). The expected supply of cobalt and selenium were thereby increased from 0.29 mg to 0.65 mg and 0.30 to 0.67 mg per head/day respectively. In those circumstances two boluses would supply more than 100% of the dietary allowances of each element for a 150 kg animal and around 60% for a 500 kg animal.

Such an increase in inclusion has therefore no adverse effects on the bolus release rate and hence the life of the bolus and gives more realistic supplements of cobalt and selenium for animals up to 500 kg.

With an increase of four fold in the inclusion rate however the bolus release rate over days 0-42 was doubled to 0.58 g/day compared to 0.28 g/day for Matrix No. 1. This was a significantly greater weight loss ($P < 0.001$) than for either of the other two formulations. Taken together with the increased inclusion rate such a bolus would supply 2.4 mg Co and 2.5 mg Se which are more than twice the total requirement for a 500 kg animal and the bolus would have a reduced expected life of 172 days. However a bolus of this formulation may be useful in circumstances of severe deficiency.

**Table 2.5.** **The effect on bolus release rate (g/bolus/day) of increased inclusion of cobalt sulphate and sodium selenite and the amounts of cobalt and selenium thus provided per day by two boluses.**

| release rate g/bolus/day | Matrix No 1 | | Increased level A 2 x Co/Se | | B 4 x Co/Se | |
|---|---|---|---|---|---|---|
| | mean | s.d. | mean | s.d. | mean | s.d. |
| **day 0-7** | 0.37 | 0.122 | 0.37 | 0.025 | 1.12 | 0.384 |
| **8-14** | 0.35 | 0.122 | 0.32 | 0.017 | 0.64 | 0.084 |
| **15-21** | 0.31 | 0.076 | 0.30 | 0.050 | 0.47 | 0.271 |
| **22-28** | 0.22 | 0.032 | 0.35 | 0.057 | 0.47 | 0.185 |
| **29-35** | 0.21 | 0.046 | 0.24 | 0.065 | 0.41 | 0.205 |
| **36-42** | 0.26 | 0.020 | 0.31 | 0.026 | 0.33 | 0.149 |
| **0-42** | **0.28** | 0.055 | **0.31** | 0.016 | **0.58** | 0.112 |
| **mean final wt (g)** | **94.5** | 4.70 | **88.2** | 0.54 | **77.6** | 4.64 |
| **expected supply mg/day** | | | | | | |
| **Cobalt** | **0.29** | | **0.65** | | **2.4** | |
| **Selenium** | **0.30** | | **0.67** | | **2.5** | |

| | | | |
|---|---|---|---|
| **Mean loss 0-42 days** | Matrix No 1 v 4xCo/Se | SED 0.045 | $P<0.001$ |
| | 2xCo/Se v 4xCo/Se | SED 0.050 | $P<0.001$ |

As with Experiment 2.4. a small change in formulation (adding 0.75 mg cobalt sulphate and 0.36 mg sodium selenite to 100 g of matrix), has had a marked effect on bolus release rate. The magnitude of this effect in absolute quantities varies with the component, e.g. increasing the quantity of zinc sulphate heptahydrate (Experiment 2.2.) in the bolus from 5 to 15% had a similar effect to increasing the cobalt sulphate and sodium selenite by less than 1% each. The reasons for this are not clear.

## Experiment 2.6

### The effect on bolus release rate (g/bolus/day) of reducing the bolus weight.

### Introduction

For the initial formulation trials the bolus weight was fixed at 100 g. This weight was close the the maximum fill of the die and was standardised at that weight so that all boluses would be equally compressed. With the daily release rate of bolus Matrix No 1 being around 0.25 g (Table 1.2.) such a bolus would last 400 days. This was considered too long a period so this experiment examined the release rate of a 75 g bolus which would be expected (if release rates were equal) to last for 300 days. This is a more realistic target for the supply of trace elements to grazing animals. With less material being placed in the die with the same pressure being applied, the compression should be greater and this may affect the release rate.

### Materials and methods

These were as in Section 1.5 with bolus Matrix No 1 except that the bolus weight was 75 g. The test period was 56 days and the cows were fed hay and 2 kg concentrates for the whole of that period.

### Results

The daily release rate of 75 g boluses are compared to the standard 100 g boluses in Table 2.6. There was no significant difference in the bolus release rates over the test period.

The results indicate the essential similarity in release rate from boluses with initial weights of either 75 or 100 g over a continuous period of 56 days. Again, the losses in weight over days 0-7 (particularly) and days 8-14 were greater than the general pattern established thereafter.

**Table 2.6. The effect on bolus release rate (g/bolus/day) of reducing the initial bolus weight.**

| | Weight | | | |
|---|---|---|---|---|
| | 75 g | | 100g* | |
| | mean | s.d. | mean | s.d. |
| **Days** | | | | |
| **0-7** | 0.61 | 0.046 | 0.37 | 0.122 |
| **8-14** | 0.31 | 0.087 | 0.35 | 0.122 |
| **15-21** | 0.17 | 0.041 | 0.31 | 0.076 |
| **22-28** | 0.20 | 0.030 | 0.22 | 0.032 |
| **29-35** | 0.15 | 0.012 | 0.21 | 0.044 |
| **36-42** | 0.20 | 0.007 | 0.26 | 0.020 |
| **43-49** | 0.19 | 0.014 | 0.18 | 0.059 |
| **50-56** | 0.18 | 0.009 | 0.24 | 0.061 |
| **mean 0-56** | **0.25** | 0.027 | **0.27** | 0.030 |
| **mean final bolus wt.** | **60.9** | 1.54 | **85.8** | 1.55 |

* from Table 1.2, Group 1.

**Mean loss 0-56 days** SED 0.014 NS

## Experiment 2.7.

## The effect on bolus release rate (g/bolus/day) of the number of resin coats applied to the bolus.

### Introduction

The standard treatment when coating the boluses with resin was to apply two coats at an interval of 4-6 hours. This was to ensure that coating was complete. Dipping twice also gave a cosmetically superior product. This experiment examines the effect on release rate of applying only a single coat of the resin.

### Materials and methods

Two boluses per animal were used and they were produced of bolus Matrix No 1 as detailed in Section 1.5 except that only a single coat of resin was applied. This was done with care to ensure that coverage was complete. The test period was 56 days and hay with 2 kg concentrate were fed for the whole of that period.

### Results

The release rate for the single coated boluses are shown in Table 2.7. Comparison was made with results from the two-coat boluses in Table 1.2. The single coated boluses were domed in appearance at the exposed end due to the bolus matrix being more resistant to erosion than the thinner coating.

Reducing the coating thickness significantly ($P < 0.001$) increased the release rate of the boluses. The initial mean loss/day over the first 7 days was 1.15 g for the single coat compared with 0.37 g for the standard double coat. After that period the release rate was between 0.5 - 0.6 g/day for 35 days, which is approximately twice that of the two coated boluses. The variation in release rate between boluses for those given a single coat was large (coefficient of variation up to 40%).

**Table 2.7.** **The effect on bolus release rate (g/bolus/day) of the number of coats of resin applied to the bolus.**

| | One coat | | Two coats* | |
|---|---|---|---|---|
| **Days** | mean | s.d. | mean | s.d. |
| **0-7** | 1.15 | 0.223 | 0.37 | 0.122 |
| **8-14** | 0.54 | 0.210 | 0.35 | 0.122 |
| **15-21** | 0.50 | 0.113 | 0.31 | 0.076 |
| **22-28** | 0.58 | 0.110 | 0.22 | 0.032 |
| **29-35** | 0.56 | 0.117 | 0.21 | 0.044 |
| **36-42** | 0.56 | 0.225 | 0.26 | 0.020 |
| **43-49** | 0.40 | 0.062 | 0.18 | 0.059 |
| **50-56** | 0.36 | 0.050 | 0.24 | 0.061 |
| **mean 0-56** | **0.58** | 0.077 | **0.27** | 0.030 |
| **mean final bolus wt.** | **67.9** | 4.25 | **85.8** | 1.55 |

* from Table 1.2, Group 1.

**Mean loss 0-56 days** SED 0.029 $P < 0.001$

## Experiment 2.8.

## The effect on bolus release rate (g/bolus/day) of the number of boluses administered.

### Introduction

From the outset of this project two boluses has been the preferred treatment since it was thought that in addition to the loss by dissolution, the abrasion of the exposed surface played an important part in the release of material from the bolus. It was also thought that the release rate of a single bolus would be prone to excessive variation due to the variable presence of grit in the reticulum. This experiment examines the effect on release rate of either a single or triple bolus treatment.

### Materials and methods

The boluses were produced as in Section 1.5 of bolus Matrix No 1 and the results for the two bolus treatment in Table 1.2 are used here to compare with the single or triple bolus administration. The test period was 42 days and hay and 2 kg concentrate were fed for the whole of that period.

### Results

The release rates are shown in Table 2.8.

As expected the presence of only a single bolus in the reticulum reduced the individual bolus release rate although this was not significantly less than when two were given. The daily loss was also more variable with the coefficient of variation for the overall release rate over days 0 to 42 being 82.3%.

With three boluses in the reticulum the release rate of the individual bolus was increased significantly from that of two boluses ($P < 0.001$), variation in release rate was reduced being only 20% for two boluses and 18% for three boluses.

The total daily release of material from all boluses present was 0.18 g for one bolus, 0.56 g for two boluses and 1.77 g when three boluses were given.

The number of boluses present in the reticulum has therefore a major effect on release rate.

**Table 2.8. The effect on bolus release rate (g/bolus/day) of the number of boluses present in the reticulum.**

| | Boluses in reticulum | | | | | |
|---|---|---|---|---|---|---|
| | One | | Two* | | Three | |
| | mean | s.d. | mean | s.d. | mean | s.d. |
| **Days** | | | | | | |
| **0-7** | 0.30 | 0.108 | 0.37 | 0.122 | 0.78 | 0.204 |
| **8-14** | 0.16 | 0.132 | 0.35 | 0.122 | 0.53 | 0.132 |
| **15-21** | 0.13 | 0.122 | 0.31 | 0.076 | 0.58 | 0.120 |
| **22-28** | 0.13 | 0.159 | 0.22 | 0.032 | 0.56 | 0.174 |
| **29-35** | 0.19 | 0.211 | 0.21 | 0.044 | 0.60 | 0.366 |
| **36-42** | 0.14 | 0.157 | 0.26 | 0.020 | 0.49 | 0.141 |
| **mean 0-42** | **0.18** | 0.144 | **0.28** | 0.056 | **0.59** | 0.108 |
| **mean final bolus wt.** | **94.5** | 4.70 | **90.0** | 2.09 | **77.3** | 4.60 |

* from Table 1.2, Group 1.

| | | | | |
|---|---|---|---|---|
| **Mean loss 0-56 days** | One v Two | SED | 0.062 | NS |
| | One v Three | SED | 0.071 | $P<0.001$ |
| | Two v Three | SED | 0.047 | $P<0.001$ |

## Experiment 2.9.

**The effect on the release rate (g/bolus/day) of the multiple trace element bolus with the presence of other boluses and their residues.**

### Introduction

The multiple trace element bolus (MTEB) is designed to erode completely to leave no permanent residue. Unfortunately this is not the case with some other bolus products which leave metallic base weights or sleeves in the reticulum once the active life of the bolus is complete.

There are currently two such products in frequent use, one releases an anthelmintic and the other a growth promoter. A third bolus which releases an anthelmintic was recently withdrawn and replaced with a trilaminate sheet which leaves no residue. This change was mainly due to opposition to the steel sleeve from abattoirs because of damage to machinery. (Zimmerman & Hoberg, 1988).

Since this particular product was to supply anthelmintic to first grazing season calves it is obviously still present in the reticulum of some adult cattle and it has been considered along with two products currently available.

In Experiment 2.8 it was shown that increasing the number of MTEB in the reticulum from 2 to 3 increased the daily release (g/bolus/day) by some 47% (Table 2.8). It was considered that the presence of other bolus products and/or their residues would have a similar or greater effect on MTEB release rates by enhancing the rate of erosion from the exposed end surface or by possible damage to the polymer coating.

This experiment examines the effect of the three bolus products and their residues on the release rate of the MTEB.

### Materials and methods

The MTEB used were the standard production ALL-TRACE boluses. These weighed 85 g and had density of 2.6 g/cm$^3$. Two were placed into the reticulum of three fistulated cows as pairs with no other bolus or residue to provide a control and then either singly or in pairs with the following other boluses and their residues, again using 3 cows per test.

The OPRB was tested both as the active bolus and the end weight residue. The MSRB was evaluated as the residual metal sleeve and the entire RDD active bolus was investigated.

1. Oxfendazole pulse release bolus (OPRB) (Autoworm, Pitman Moore Ltd)
2. ORPB end weight consisting of a steel cylinder
3. Morantel sustained release bolus metallic sleeve (MSRB) (Paratect, Pfizer Ltd)
4 Romensin rumen delivery device (RDD) (Romensin RDD Elanco Products Ltd)

The weight and dimensions of these were:

| | weight (g) | diameter (mm) | length (mm) | density $(g/cm^3)$ |
|---|---|---|---|---|
| 1. OPRB | 130 | 25 | 90 | 3.0 |
| 2 OPRB end weight | 90 | 25 | 25 | 7.5 |
| 3 MSRB sleeve | 102 | 25 | 87 | 7.6 |
| 4 RDD | 240 | 35 | 110 | 3.4 |

Plates 6, 7 and 8 show respectively the oxfendazole pulse release bolus, the romensin rumen delivery device, the morantel sustained release bolus and the permanent residues which remain after their active life.

In each case the test period was 14 days and the cows were fed silage ad libitum over that period. The release rates were monitored as detailed in Section 1.5.

**Results**

The results are given in Table 2.9 which details the release rate per bolus (g/day) over the 14-day test period. The mean release rate for the pair of MTEB was 0.35 g/day per bolus. In contrast the release rate of the MTEB when metallic bolus or its residue was present was generally some ten fold greater. The variation found within each treatment was also very considerable e.g. for the single MTEB in the presence of one entire OPRB the coefficient of variation was 4% but when two MTEBs were given it was 30%. In contrast, in the presence of the RDD the values were 38% for the single MTEB and 7% for a pair of MTEBs.

**Table 2.9. The effects on the bolus release rate (g/bolus/day over days 0-14) of the presence of alternative bolus products and the number of MTEB administered simultaneously.**

| Bolus | MTEBs given | Release rate g/bolus/day mean | s.d. | Residue (g) mean | s.d. | **Projected life (days)** |
|---|---|---|---|---|---|---|
| OPRB | 1 | 4.42 | 0.175 | 22.4 | 2.24 | **19** |
| | 2 | 4.07 | 1.240 | 27.6 | 17.35 | **21** |
| OPRB | 1 | 3.37 | 1.113 | 39.1 | 15.74 | **25** |
| end weight | 2 | 4.15 | 0.810 | 29.5 | 10.02 | **20** |
| MSRB | 1 | 1.10 | 0.365 | 61.8 | 8.12 | **77** |
| sleeve | 2 | 5.79 | 0.413 | 11.3 | 1.93 | **15** |
| RDD | 1 | 2.92 | 0.120 | 45.3 | 17.50 | **29** |
| | 2 | 24.94 | 0.362 | 16.9 | 5.34 | **17** |
| MTEB alone as control | 2 | 0.35 | 0.07 | 82.5 | 0.55 | **243** |

**Discussion**

If the multiple trace element bolus is to be given simultaneously or subsequent to other devices which leave permanent residues in the rumen then quite unpredictable results may be expected. Consideration might then be given to the administration of a single MTEB bolus followed by another after an interval which can only be estimated at 75-80 days. The animal would of course obtain benefit from that part of the more rapidly than normal release of material (e.g copper and selenium) which can be stored in the body of the animal.

**Plate 6.**
**The oxfendazole pulse release bolus and the end weight which remains as a permanent residue.**

**Plate 7.**
**The romensin rumen delivery device and the steel sleeve which remains as a permanent residue.**

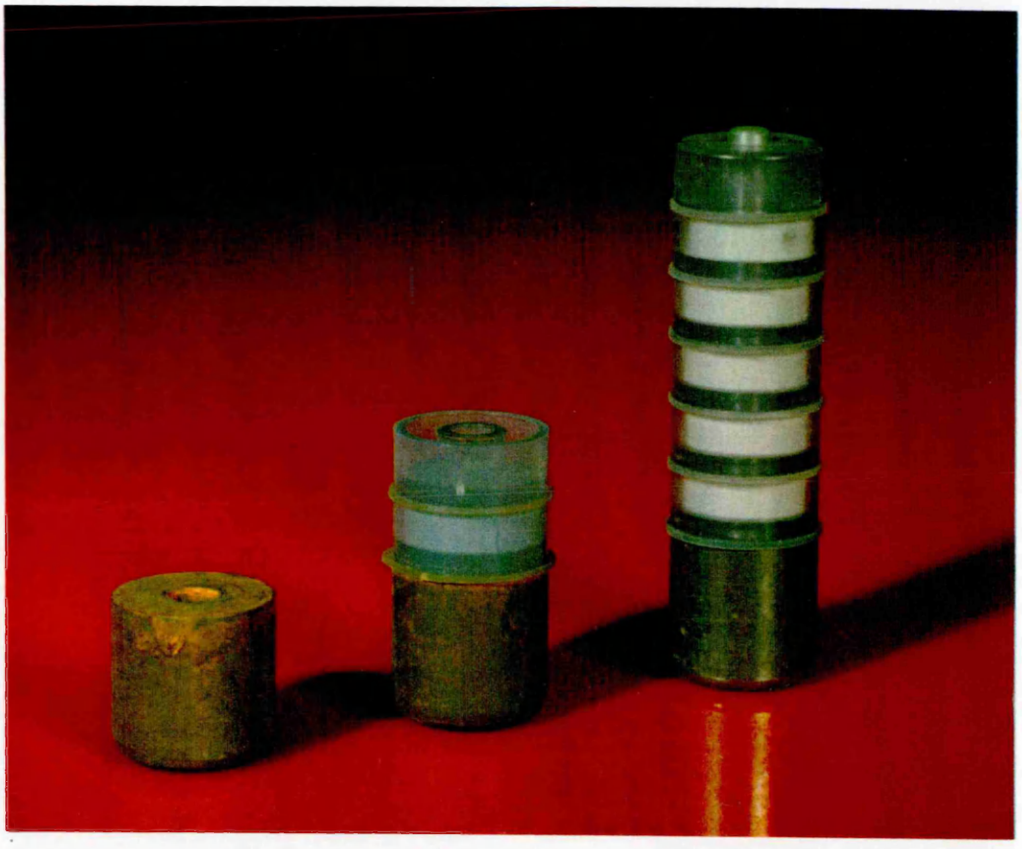
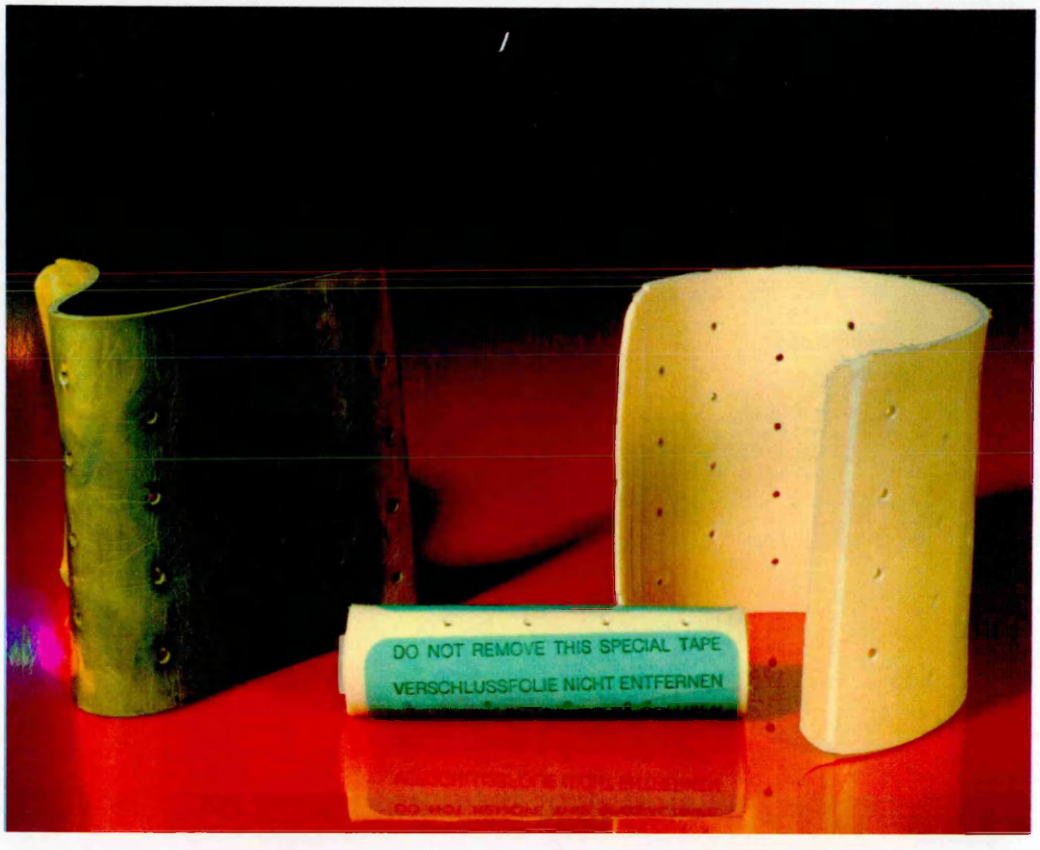


**Plate 8.**
**The morantel sustained release bolus and the steel sleeve which remains as a permanent residue.**

**Plate 9.**
**The morantel trilaminate plastic sheet which is rolled up for administration and then unfolds within the reticulo-rumen. The discoloured sheet was recovered at slaughter.**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## Experiment 2.10.

## A survey of the number and type of bolus residues found in cattle at slaughter.

### Introduction

Experiment 2.9 demonstrated that the presence of the permanent metallic residues remaining in the reticulo-rumen from other bolus products could reduce the projected life of the MTEB bolus to as short a period as 15 days. This was presumably due to the abrasive action of these varied metallic objects. To discover the extent of this problem a limited survey was carried out in a commercial abattoir to determine the numbers and type of bolus residues found in cattle at slaughter.

### Materials and methods

The survey was carried out over the period 1 October to 19 October 1990. A detailed record was kept of the number of cattle slaughtered at the abattoir. The reticulo-rumen of all cattle were searched and any bolus residues found were removed, washed and identified. All boluses were required to be removed by hand to avoid damage to abattoir machinery.

### Results

### Number of cattle

Over the twenty day survey period 2521 cattle were slaughtered at the abattoir. This was composed of 380 different groups of cattle consigned by five wholesale and twenty-six retail butchers. The largest group was of 20 animals but 92% of consigned groups were of between 4 and 10 cattle.

The animals were predominantly finished heifers and bullocks but there were 154 bull-beef type animals also consigned for slaughter. They were bought from auction markets throughout Scotland and the north of England as well as direct from farms.

**Number and type of bolus residue**

A total of 435 bolus residues were recovered from the 2521 cattle slaughtered. Since the bolus residues were recovered by abattoir personnel and collected at the end of the daily slaughter period it can only be assumed that each bolus residue was found singly. On this assumption about 17% of young cattle slaughtered from grass contain bolus residues. Table 2.10 gives the breakdown of this total figure and Table 2.11 gives the dimensions of the major bolus residues found.

**Table 2.10 Number and type of bolus residues recovered from cattle at slaughter over a three week period.**

| | |
|---|---|
| Romensin RDD | 162 |
| OPRB | 143 |
| Morantel 'tube' | 57 |
| Morantel 'flex' | 61 |
| Cobalt bullet | 7 |
| Cosecure glass bolus | 4 |
| All-trace | 1 |
| Total | 435 |

The Romensin RDD (Elanco Products Ltd.) was found in the largest numbers (162) with the oxfendazole pulse release bolus OPRB (Autoworm, Coopers Pitman Moore, Synthantic multidose, Syntex Animal Health) being the anthelmintic bolus found in the greatest numbers (143). One OPRB residue still had one tablet remaining enclosed within the plastic segments. The morantel sustained release bolus MSRB (Paratect, Pfizer Ltd.) metal tube and the trilaminate plastic sheet (which replaced the metal tube type) were found in approximately equal numbers (57 versus 61). The trilaminate sheet in contrast to the other 3 major boluses was designed to disintegrate leaving no residue. Plate 9 shows the morantel trilaminate sheet rolled up ready for administration and unrolled as it is within the reticulo-rumen. Over 98% of those recovered in this study were completly intact with the majority folded in half. Only one sheet showed signs of cracking from the holes within the sheet and another was roughly half size having been split vertically.

**Table 2.11 Weight (g) and dimensions (mm) of the major residues recovered.**

| | weight | diameter | length | width |
|---|---|---|---|---|
| Romensin RDD | 198 | 35 | 110 | - |
| OPRB base weight | 90 | 25 | 25 | - |
| Morantel tube | 102 | 25 | 87 | - |
| Morantel flex | 45 | - | 210 | 98 |

The four major bolus residues recovered accounted for 97% of the total. The other 3% consisted of boluses given as trace-element supplements. Seven cobalt bullets (Permaco-C, Coopers Pitman-Moore Ltd.) were found. No sign of surface coating was observed. Four soluble glass boluses (COSECURE, Coopers Pitman-Moore Ltd.) were also found. These were of the original type withdrawn because of irregular dissolution. Those found weighed a mean of 5 g less than an unused sample. One MTEB was recovered (All-trace, Agrimin Ltd.). It weighed 47 g and was of the lower density type which may explain the absence of the second bolus.

**Discussion**

With over 2500 cattle consigned to the abattoir for slaughter over the period and from a wide range of sources it is assumed that the results are representative of cattle from south/south-west Scotland. Using the assumption outlined earlier, of this large number of cattle 17% contained a bolus residue and 15% contained a permanent metallic residue. At this level there appears to be a significant problem for the use of the MTEB bolus, especially since those farmers using one bolus system are perhaps more likely to use another bolus system either consecutively or at another stage in the productive life of the animals.

Observations from this and other work indicates that often two or more boluses are present in the reticulo-rumen. Investigation of this and the effect that numerous metallic residues would have on the reticulum could be the basis for a more detailed study. Clearly, it is desirable that no permanent residues should remain after any bolus treatment.

## Development and testing of the multiple trace element bolus as a commercial product.

### Introduction

The results from the experiments described earlier in this Section demonstrated that administration of two boluses using Matrix No 1 would give a release rate of around 0.25 g/bolus/day and that this could be maintained at a reasonably constant rate over long periods of time. The release rate of the bolus was independent of dietary changes and could be altered by changes in formulation of the bolus matrix or by other factors such as coating and the number of boluses administered. These encouraging results suggested that it should be possible to design a bolus to supply appropriate amounts of six trace elements (copper, cobalt, selenium, iodine, zinc and manganese) to be of benefit to cattle grazing herbage considered to be of inadequate trace element status. There would be a substantial demand for such a proprietary product.

The profile for such a product would be that it supply at least 50% of the three major trace elements copper, cobalt and selenium for cattle up to 500 kg liveweight. This was set to supplement the levels of trace elements supplied by herbage classed as deficient. The product should supply these quantities over a 300 day period. It was envisaged that two boluses would be administered using an oesophageal balling gun on transfer to grass in the spring.

### Changes in bolus weight and formulation

The manufactured bolus had a reduced weight of 85 g rather than the 100 g prototype bolus since the life of the bolus was to be reduced. This could be done without affecting the release rate (Experiment 2.6) and the length would be reduced.

Table 2.12 details the trace element and vitamin content of bolus Matrix No 1 and the amounts of each element provided per day assuming two boluses lose a combined 0.5 g matrix per day.

**Table 2.12 Trace element and vitamin supply from two boluses of Matrix No 1.**

| Component | Inclusion in matrix g/100 g | % Nutrient content | mg nutrient per 0.5 g bolus matrix |
|---|---|---|---|
| CuO | 25.0 | 77 | 97 |
| $MnSO_4$ $H_2O$ | 33.38 | 31 | 52 |
| $ZnSO_4$ $7H_2O$ | 16.67 | 22 | 19 |
| ZnO | 16.89 | 80 | 68 |
| $CoSO_4$ $7H_2O$ | 0.25 | 21 | 0.25 |
| $Na_2SeO_3$ | 0.12 | 45 | 0.25 |
| KI | 0.45 | 68 | 1.55 |
| Vit A/D3 | 3.62 | 500,000 iu Vit A/g<br>100,000 iu Vit D/g | 9000 iu Vit A<br>1800 iu Vit D3 |
| Vit E | 3.62 | 50 | 9 |

These amounts have to be considered in relation to the dietary trace element allowances (i.e. dietary minimum trace element allowances as proposed by ARC (1980) plus appropriate safety factors). Those adopted for present purposes are those detailed by a Joint Working Party composed of the Ministry of Agriculture, Fisheries and Food, Department of Agriculture for Scotland, Department of Agriculture for Northern Ireland, United Kingdom Agricultural Supply Trade Association and the British Veterinary Association (MAFF et al, 1983). These were (mg/kg DM) Cu 12, Co 0.11, Se 0.10, I 0.15, Zn 40 and Mn 40. Table 2.13 details these dietary allowances for three classes of cattle 150, 300 and 500 kg representing respectively young cattle going to grass, yearling heifers and suckler cows. It is assumed that their dry matter intakes would be 5, 7.5 and 10 kg/day respectively.

**Table 2.13 The recommended daily dietary allowances (mg) of trace elements and vitamins (iu's) for cattle (MAFF et al, 1983).**

| Liveweight kg | 150 | 300 | 500 |
|---|---|---|---|
| Assumed DM intake kg | 5 | 7.5 | 10 |
| Provided by two Matrix No 1 boluses (Table 2.12) | | | |
| Copper | 97 | 60 | 90 | 120 |
| Cobalt | 0.25 | 0.55 | 0.83 | 1.1 |
| Selenium | 0.25 | 0.5 | 0.75 | 1.0 |
| Iodine | 1.55 | 0.75 | 1.13 | 1.50 |
| Manganese | 52 | 200 | 300 | 400 |
| Zinc | 87 | 200 | 300 | 400 |
| Vitamin A (iu) | 9000 | 10,000 | 20,000 | 50,0000 |
| Vitamin D3 (iu) | 1800 | 900 | 1800 | 5,000 |
| Vitamin E | 9 | 75 | 113 | 150 |

Two boluses with a combined release rate of 0.5 g/day would supply 81%, 23% and 25% of the copper, cobalt and selenium recommended daily allowances for 500 kg cattle. Increased inclusion levels of cobalt sulphate and sodium selenite were therefore required to meet the 50% target for 500 kg cattle. Experiment 2.5 showed that the amounts in bolus Matrix No 1 could be doubled without affecting bolus release rate.

For 500 kg cattle the amounts of Vitamins A and $D_3$ were considerably less than the full requirements and for Vitamin E the contribution from bolus Matrix No 1 was extremely small. Since the Vitamin A/D3 to be supplied by Roche Products Ltd would in future be Type P the inclusion of Vitamin A/$D_3$ was reduced by half. The results of Experiment 2.4 suggested that a reduction to 25% of the original inclusion in Matrix No 1 would be required to reduce the release rate to less than 0.5 g/bolus/day. It was however considered that a reduction to this degree would result in too low a provision of vitamins. In consequence Vitamin A/$D_3$ Type P was only reduced to 50% of the original inclusion.

To overcome the expected slighly greater release rate of such a formulation (Table 2.4) the inclusion of copper oxide was increased as this had been shown to reduce the release rate (Table 2.3)

For convenience of presentation the modified Matrix No 1 was termed 'All-trace' (the name under which the product was eventually available commercially). Comparisons of the separate inclusions of the various components are given in Table 2.14.

**Table 2.14 The formulation for 'All-trace' compared with Matrix No 1.**

| Component % | Matrix No 1 | 'All-trace' | Change (%) |
|---|---|---|---|
| CuO | 25.00 | 26.50 | +6 |
| $MnSO_4$ $H_2O$ | 33.38 | 33.45 | 0 |
| $ZnSO_4$ $7H_2O$ | 16.67 | 16.67 | 0 |
| ZnO | 16.89 | 16.88 | 0 |
| $CoSO_4$ $7H_2O$ | 0.25 | 0.50 | +100 |
| $Na_2SeO_3$ | 0.12 | 0.25 | +108 |
| KI | 0.45 | 0.46 | +2 |
| Vit $A/D_3$ | 3.62 | 1.77 | -51 |
| Vit E | 3.62 | 3.53 | 0 |

**Plate 10**

**A pair of commercially manufactured mutliple trace element boluses (MTEB).**

**Experiment 2.11**

**Evaluation of the 'All-trace' bolus in rumen-fistulated cows.**

**Introduction**

In contrast to the laboratory preparation of boluses used in the initial trials which employed a single-end press, the manufacture of the commercial 'All-trace' bolus which was carried out by Rumbol Products Ltd used a double-ended press. A representative batch was supplied to the University of Glasgow for testing in fistulated cows. The boluses were 62 mm in length, 26 mm in diameter, had a density of 2.6 g/cm$^3$ and the uncoated end of each bolus was temporarily sealed with a wax coating which was removed rapidly in the rumen.

**Materials and Methods**

These were as in Section 1.5. for bolus testing, except that pairs of boluses were used in each of six fistulated cows. The test period was 63 days and the cows were at grass for the whole of that period.

**Results**

The overall mean release rate over days 0-63 was 0.43 g/bolus/day which was about 70% greater than for Matrix No 1. It was anticipated that this might be the case due to the inclusion level of Vitamin A/D$_3$ type P. Over days 0-7 0.98 g/bolus/day was lost and this fell to 0.56 g/bolus/day in the second week. The release rate fell more slowly over days 22 to 63 from 0.37 g/bolus/day to 0.28 g/bolus/day (Table 2.15).

If it is assumed that the release rate will remain at that level then the expected life of the bolus is about 240 days. This is considerably less than the initial target of about 300 days but it was considered that an eight month period would adequately cover a normal grazing period.

**Table 2.15 The release rate (g/bolus/day) of 'All-trace' boluses when placed in pairs into six fisulated cows.**

| Days | mean | s.d. |
|---|---|---|
| **0-7** | 0.98 | 0.153 |
| **8-14** | 0.56 | 0.114 |
| **15-21** | 0.40 | 0.084 |
| **22-28** | 0.37 | 0.059 |
| **29-35** | 0.35 | 0.053 |
| **36-42** | 0.34 | 0.032 |
| **43-49** | 0.32 | 0.027 |
| **50-56** | 0.29 | 0.023 |
| **57-63** | 0.28 | 0.058 |
| **0-63** | **0.43** | 0.031 |
| **mean final wt. (g)** | **58.2** | 2.79 |

**Assessment of the trace element contribution from the 'All-trace' bolus to the nutrient intake of cattle grazing herbage of inadequate trace element content.**

## Introduction

With the release rate altered from that of bolus Matrix No 1 it was necessary to reassess the supply of trace elements from the 'All-trace' bolus. Since the use was envisaged to be in the grazing animal it was thought that some account should be taken of the trace element concentrations that inadequate herbage would provide.

Table 2.16 shows the MAFF et al. (1983) recommended concentrations of trace elements in pasture which are adequate to meet the dietary allowance. Table 2.16 also gives the concentrations of each element which are probably the lowest likely to be encountered under conditions of "deficient" grazing (Hemingway, 1988; MacPherson, 1988; Mills, 1988).

**Table 2.16 Recommended (MAFF et al., 1983) and deficient concentrations of trace elements in herbage (mg/kg DM).**

| | Recommended concentration mg/kg | Deficient herbage mg/kg | % of Recommended |
|---|---|---|---|
| Copper | 12 | 3 | 25 |
| Cobalt | 0.11 | 0.04 | 36 |
| Selenium | 0.10 | 0.03 | 30 |
| Iodine | 0.15 | 0.10 | 67 |
| Manganese | 40 | 30 | 50 |
| Zinc | 40 | 30 | 50 |

The concentrations considered to be deficient range from about one-quarter to two-thirds of the recommended level. Obviously with copper and iodine, antagonist compounds may limit the availability of these to the animal and complicate this perhaps over-simplified approach.

Table 2.17 gives the trace element and vitamin provision from two boluses over a period of 240 days. Table 2.18 combines that information with the contributions from deficient herbage and compares the total supplied with the dietary allowances (MAFF et al., 1983) for 150, 300 and 500 kg cattle.

**Table 2.17 The supply of trace elements and vitamins from two 'All-trace' boluses.**

| | Total content of each 85 g bolus | Estimated provision daily for 8 months by two boluses |
|---|---|---|
| Copper | 19,200 mg | 160.0 mg |
| Cobalt | 87 mg | 0.7 mg |
| Selenium | 93 mg | 0.8 mg |
| Manganese | 8,800 mg | 74.0 mg |
| Zinc | 14,200 mg | 118.0 mg |
| Iodine | 264 mg | 2.2 mg |
| Vitamin A | 583,100 iu | 4,859.0 iu |
| Vitamin D3 | 116,620 iu | 972.0 iu |
| Vitamin E | 1,660 iu | 9.7 iu |

This data is also shown in Figures 2.2 -2.6 for the individual trace elements.

For copper the amount supplied by the boluses is in excess of total dietary needs. This is considered necessary in situations where elevated sulphur, molybdenum or iron may reduce copper availability.

For both cobalt and selenium two boluses alone provided more than the total needs of a 150 kg animal and with presumed contributions of 0.4 mg Co and 0.3 mg Se/day from deficient herbage would meet the needs of 500 kg cattle.

For zinc and manganese the combination of those amounts provided by two boluses and deficient herbage were in excess of requirements for 150 and 300 kg cattle and approximated to the needs of 500 kg cattle consuming 10 kg herbage dry matter.

Two boluses provided considerably more iodine than the dietary allowance for all liveweights of cattle.

These calculations are based on the mean release of the bolus matrix when two boluses were evaluated in cows with a rumen fistula. The experiments described in Section 3 were conducted to assess the adequacy in more realistic field conditions, frequently where there was a past history of trace element inadequacy.

In situations where a greater supply of trace elements might be required e.g. extremely deficient herbage, for cattle of higher liveweights or for higher producing animals more than two boluses could be given.

**Table 2.18 Assessment of total nutrient intake (mg/day) from two boluses plus deficient herbage when compared to the dietary allowance for 150 kg, 300 kg and 500 kg cattle.**

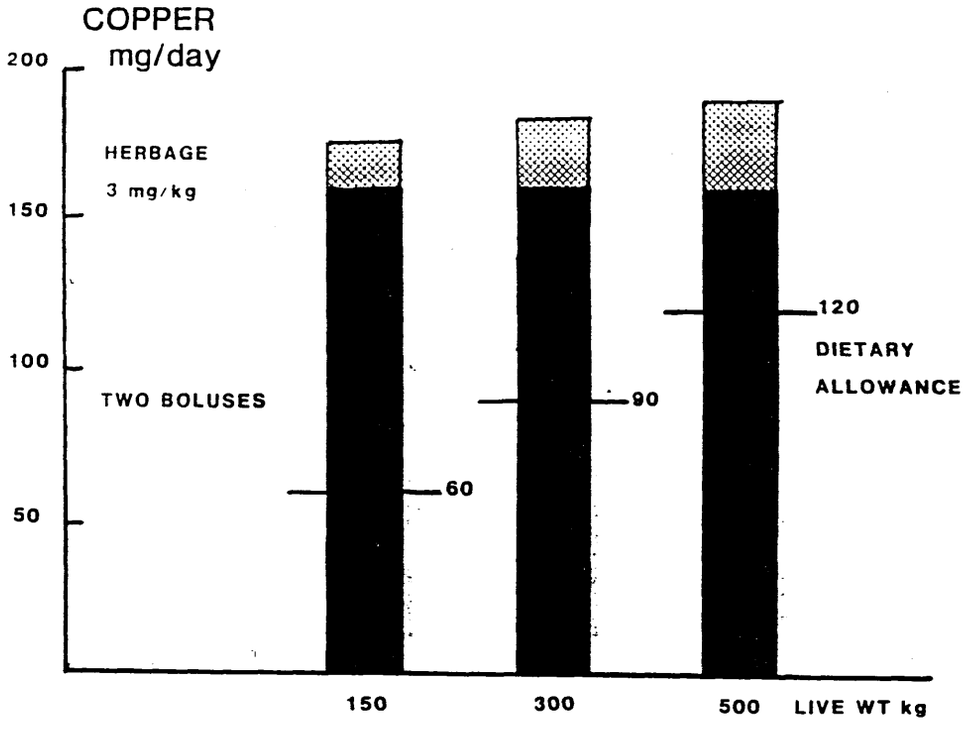
| | 150 kg | 300 kg | 500 kg |
|---|---|---|---|
| Assumed DM intake (kg) | 5 | 7.5 | 10 |
| **COPPER** | | | |
| Deficient herbage | 15 | 22.5 | 30 |
| Two boluses | 160 | 160 | 160 |
| Total | **175** | **182.5** | **190** |
| Dietary allowance | **60** | **90** | **120** |
| **COBALT** | | | |
| Deficient herbage | 0.2 | 0.3 | 0.4 |
| Two boluses | 0.7 | 0.7 | 0.7 |
| Total | **0.9** | **1.0** | **1.1** |
| Dietary allowance | **0.55** | **0.83** | **1.1** |
| **SELENIUM** | | | |
| Deficient herbage | 0.15 | 0.23 | 0.3 |
| Two boluses | 0.8 | 0.8 | 0.8 |
| Total | **0.95** | **1.03** | **1.1** |
| Dietary allowance | **0.5** | **0.75** | **1.0** |
| **MANGANESE** | | | |
| Deficient herbage | 150 | 225 | 300 |
| Two boluses | 74 | 74 | 74 |
| Total | **224** | **299** | **374** |
| Dietary allowance | **200** | **300** | **400** |
| **ZINC** | | | |
| Deficient herbage | 150 | 225 | 300 |
| Two boluses | 118 | 118 | 118 |
| Total | **268** | **343** | **418** |
| Dietary allowance | **200** | **300** | **400** |
| **IODINE** | | | |
| Deficient herbage | 0.5 | 0.75 | 1.0 |
| Two boluses | 2.2 | 2.2 | 2.2 |
| Total | **2.7** | **2.95** | **3.2** |
| Dietary allowance | **0.75** | **1.13** | **1.50** |

**Figure 2.1**

**Comparison of the copper supplied by two mutliple trace element boluses plus inadequate herbage with the dietary allowance for 150, 300 and 500 kg cattle.**

**Figure 2.2**

**Comparison of the cobalt supplied by two mutliple trace element boluses plus inadequate herbage with the dietary allowance for 150, 300 and 500 kg cattle.**


COPPER
mg/day
200
150
100
50
HERBAGE
3 mg/kg
TWO BOLUSES
60
90
120
DIETARY
ALLOWANCE
150
300
500
LIVE WT kg

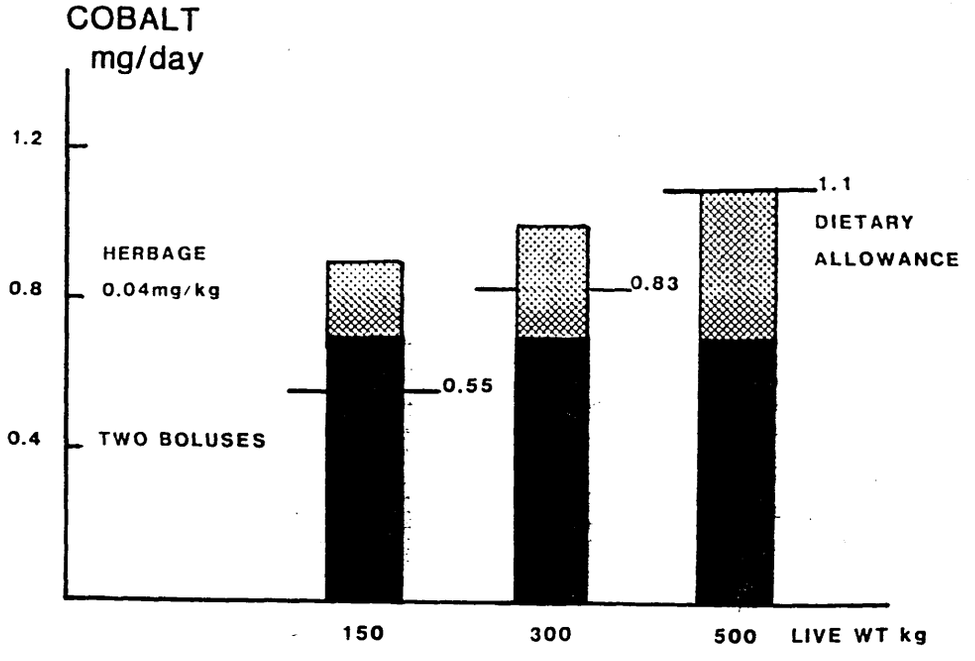
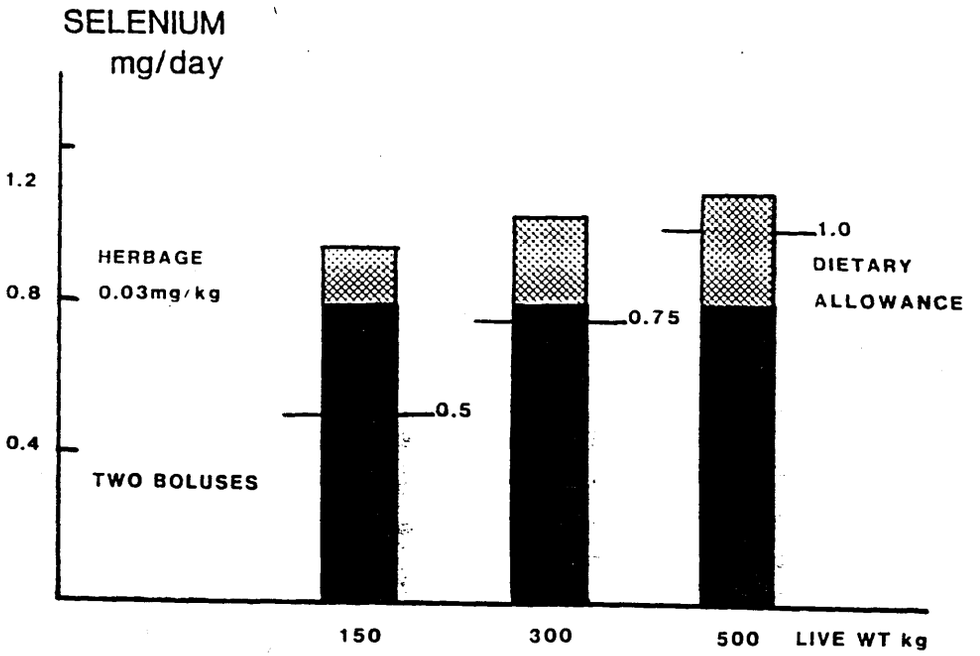

COBALT
mg/day
1.2
0.8
0.4
HERBAGE
0.04mg/kg
TWO BOLUSES
0.55
0.83
1.1
DIETARY
ALLOWANCE
150
300
500
LIVE WT kg

**Figure 2.3**

**Comparison of the selenium supplied by two mutliple trace element boluses plus inadequate herbage with the dietary allowance for 150, 300 and 500 kg cattle.**

**Figure 2.4**

**Comparison of the iodine supplied by two mutliple trace element boluses plus inadequate herbage with the dietary allowance for 150, 300 and 500 kg cattle.**


SELENIUM
mg/day
1.2
0.8
0.4
HERBAGE
0.03mg/kg
TWO BOLUSES
0.5
0.75
1.0
DIETARY
ALLOWANCE
150
300
500
LIVE WT kg

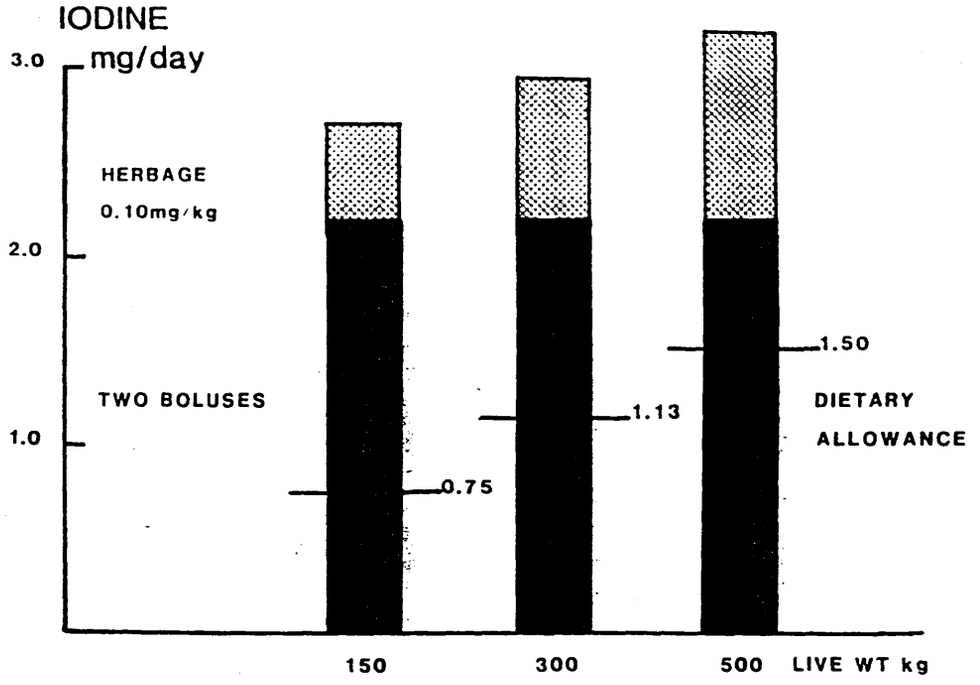
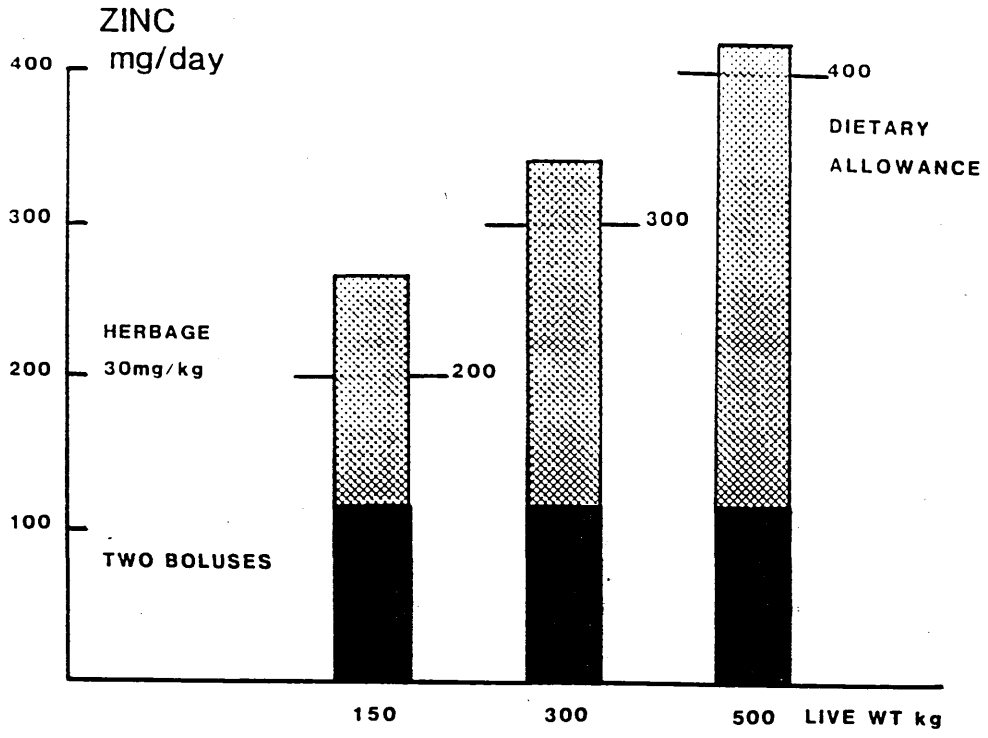

IODINE
3.0
mg/day
HERBAGE
0.10mg/kg
2.0
TWO BOLUSES
1.0
0.75
1.13
1.50
DIETARY
ALLOWANCE
150
300
500
LIVE WT kg

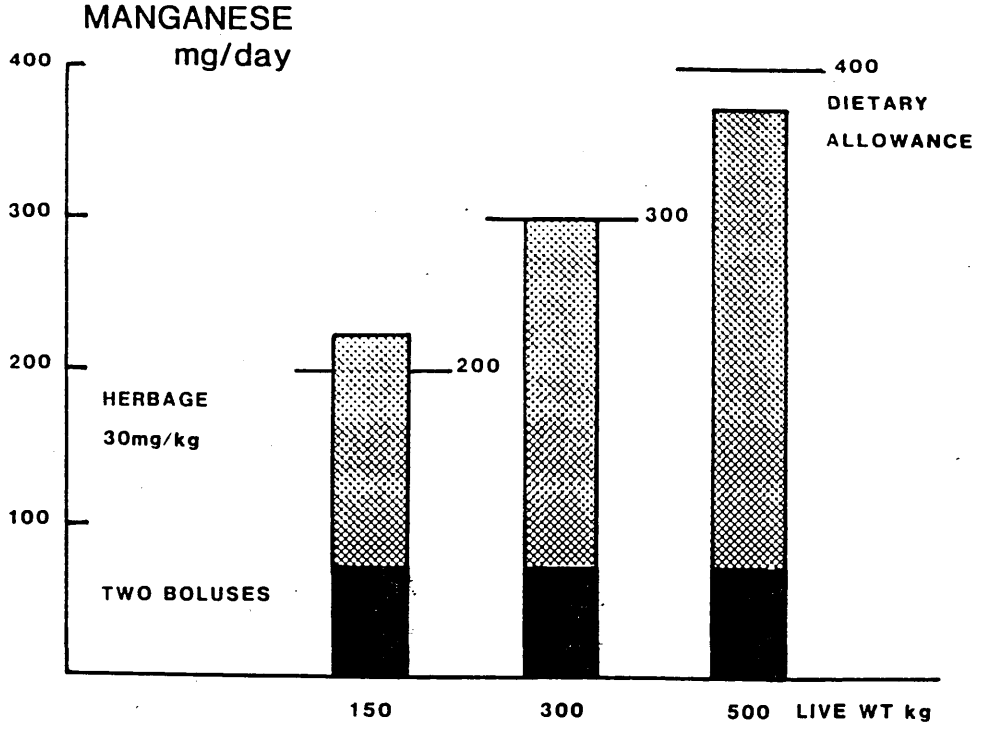
**Figure 2.5**

**Comparison of the zinc supplied by two mutliple trace element boluses plus inadequate herbage with the dietary allowance for 150, 300 and 500 kg cattle.**

**Figure 2.6**

**Comparison of the manganese supplied by two mutliple trace element boluses plus inadequate herbage with the dietary allowance for 150, 300 and 500 kg cattle.**


ZINC
mg/day
400
300
200
100
HERBAGE
30mg/kg
TWO BOLUSES
200
300
400
DIETARY
ALLOWANCE
150
300
500
LIVE WT kg


MANGANESE
mg/day
400
300
200
100
HERBAGE
30mg/kg
TWO BOLUSES
200
300
400
DIETARY
ALLOWANCE
150
300
500
LIVE WT kg

## Experiment 2.12

**An assessment of the uniformity of individual composition of boluses within a particular production batch both before and after administration to cattle.**

### Introduction

Visual inspection of very many individual boluses on removal from the rumen indicates a change in colour (generally a darkening) of the surface extending sometimes to 1 - 2 mm depth. The surfaces are invariably intact and with no indication of cracking or crumbling. When the polymer coating is removed there are no obvious visual signs of change throughout the length of the bolus.

Loss of material is by a combination of erosion and solution. The various ingredients differ greatly in their immediate solubility. It is important to confirm that loss of ingredients is uniform and that there is no rapid removal of the more soluble components and particularly of those present in smaller amounts such as cobalt and selenium.

It is also important to establish the uniformity of composition of individual boluses within a manufacturer's batch to ensure that the minor ingredients are uniformly distributed between boluses.

### Materials and methods

One full box containing twenty boluses was selected at random from a production run of several hundred boluses which had been packed at random into boxes each of twenty boluses. Four boluses were selected at random from the box of twenty for initial analysis.

A further four boluses were selected at random and placed together in the reticulum of one fistulated cow. They remained together in the reticulum until about one-half of each bolus had eroded/dissolved. All four were removed at the same time.

A cross-section about 4 mm thick was obtained from each of the four original and each of the four worn boluses. In each case this was taken at a depth of about 1 cm below the original or worn active end of the bolus to avoid any surface irregularities.

The sections of the boluses were dried at 50°C for two days to minimise any loss of water of hydration from the ingredients of the bolus matrix. The dried sections were analysed for copper, zinc, manganese, cobalt and selenium.

**Results**

The individual and mean concentrations with their appropriate standard deviations and standard errors are given in Table 2.19.

For the four original boluses the coefficients of variation (CV%) (i.e. standard deviation as % of the mean) were below 2.5 for weight and copper and manganese concentrations. They were between 6.5 and 9.0 for zinc, cobalt and selenium. These would all be reduced by a factor of 1.414 when two boluses were used together and were considered to be satisfactorily low.

The CV for the weight of the boluses removed from the reticulum was 7.4 (or 5.2 if a pair of boluses was considered). The CV's in respect of copper, zinc, manganese, cobalt and selenium concentration were all in the very satisfactorily low range of 1.3 to 4.3 (or 0.9 to 3.0 if a pair of boluses was considered).

In each case the mean concentration in the worn boluses was in the range 92.0 to 102.1% of that in the original bolus. For three of the five ingredients examined, zinc, cobalt and selenium, there was no significant difference in the concentration of each element in the original and worn boluses.

For both copper and manganese the mean concentrations in the worn boluses were significantly less than those in the original. However the differences were quite small, the mean concentrations in the worn boluses being 94.3 and 92.2% of the original respectively.

There is no obvious reason for these differences in respect of copper and manganese. Copper oxide is the least soluble ingredient, but the most dense. It is thus less likely to be selectively soluble but perhaps the most difficult (of the major ingredients copper oxide, manganese sulphate and zinc oxide/sulphate) to incorporate uniformly. The very low CV value for copper concentration in the original boluses (0.8%) suggests that satisfactory incorporation of copper was achieved. It could perhaps be speculated that cutting the slices of boluses for analysis caused some irregular loss of copper oxide as dust.

On the other hand, manganese sulphate is one of the major and more soluble ingredients and if there were preferential solubility then some selective loss might be expected.

**Table 2.19** **The weights of the original boluses and the worn boluses removed from the reticulum and their composition.**

| | Weight g | Copper g/kg | Zinc g/kg | Manganese g/kg | Cobalt mg/kg | Selenium mg/kg |
|---|---|---|---|---|---|---|
| **Original boluses** | | | | | | |
| | 83.9 | 220.1 | 164.7 | 114.8 | 1260 | 952 |
| | 82.5 | 231.5 | 172.4 | 111.5 | 1390 | 851 |
| | 83.3 | 230.5 | 143.3 | 110.3 | 1260 | 835 |
| | 84.0 | 230.9 | 172.6 | 110.7 | 1110 | 931 |
| Mean | 83.4 | 228.3 | 163.3 | 111.8 | 1255 | 892 |
| S. dev | 0.69 | 5.5 | 13.8 | 2.0 | 115 | 58 |
| S. dev as % mean | 0.8 | 2.4 | 8.5 | 1.8 | 9.0 | 6.5 |
| S. error of mean | 0.34 | 2.7 | 6.9 | 1.0 | 57 | 29 |
| **Worn boluses** | | | | | | |
| | 44.7 | 218.5 | 175.8 | 103.7 | 1200 | 843 |
| | 41.1 | 215.3 | 167.4 | 104.7 | 1190 | 842 |
| | 39.2 | 210.5 | 158.2 | 101.7 | 1100 | 896 |
| | 46.1 | 216.9 | 165.7 | 102.5 | 1130 | 823 |
| Mean | 42.8 | 215.3 | 166.8 | 103.2 | 1155 | 851 |
| S. dev | 3.18 | 3.5 | 7.2 | 1.3 | 48 | 31 |
| S. dev as % mean | 7.42 | 1.6 | 4.3 | 1.3 | 4.2 | 3.7 |
| S. error of mean | 1.60 | 1.7 | 3.6 | 0.7 | 24 | 16 |
| Significance of difference between means P< | NA | 0.01 | 0.67 | 0.01 | 0.18 | 0.28 |
| Worn bolus mean as % original bolus mean | 51.3 | 94.3 | 102.1 | 92.2 | 92.0 | 95.4 |

NA Not applicable.

The generally lower concentration of all ingredients in the worn boluses (apart from zinc) might result from the drying technique adopted. It is possible that the worn boluses contained more water than the original boluses following residence in the rumen. Drying at 50$^{o}$C to minimise possible loss of water of hydration of some of the ingredients may have left more residual water in the worn boluses and hence led to a general reduction in apparent concentration of all the ingredients.

In each case the difference in concentration of all the elements (except zinc) in the worn boluses was very small in relation to that in the original. Nevertheless, it was not considered that any selective erosion/dissolution occurred which would be of any practical importance to livestock given the two-bolus treatment.

In a subsequent experiment to be described (Experiment 4.4) an anthelmintic (oxfendazole) was incorporated within a range of modified bolus matrices, all of which contained copper oxide, zinc oxide/sulphate and manganese sulphate. Visual inspection of the boluses at intervals on removal from the reticulum showed no indications of selective removal of ingredients other than a change in colour of the immediate surface as erosion/dissolution proceeded. Analysis of separate 3 mm transverse slices taken along the length of the bolus following a period of immersion in the reticulum (undertaken by Syntex Ltd.) revealed no selective loss of oxfendazole in that the concentration was uniform throughout the bolus length.

**Comparison with the Declared Composition and the Analytical Composition of the Bolus.**

The Feedingstuffs Regulations (1988) would class the bolus as a 'mineral feeding stuff' which means a 'complementary feeding stuff which is composed mainly of minerals and which contains at least 40% by weight of ash'. As such 'limits of variation' are allowed between the declared composition (as calculated from the ingredient inclusions) and the actual analyses for each element. These maximum limits of variation are $\pm$ 30% of the amount stated for copper and $\pm$ 50% of the amounts stated for all the other elements in the bolus.

**Table 2.20 The declared composition and the mean analysed composition of the trace element bolus.**

| | Declared[+] | Analysed[++] | % Variation Analysed ÷ Declared |
|---|---|---|---|
| Copper g/kg | 226 | 228 | + 0.9 |
| Zinc mg/kg | 1023 | 1225 | + 19.7 |
| Manganese mg/kg | 1094 | 892 | - 18.2 |
| Cobalt mg/kg | 104 | 112 | + 7.6 |
| Selenium mg/kg | 167 | 163 | - 2.5 |

[+] Calculated from descriptive Alltrace leaflet.
[++] From Table 2.19.

Table 2.20 indicates the % variation for each of the elements. In each case the analytical composition falls well within the permitted limits of variation. For copper, cobalt and selenium the variations between the declared calculated composition and the analysed values are particularly low. It is considered that these results are satisfactory in this instance but that a system of routine checking should be adopted following an assessment of an appropriate method of sampling from a large batch of boluses.

## SECTION 3

### Field trials with the multiple trace element bolus.

This section describes a series of field trials in which two multiple trace element boluses (MTEB) were given to cattle. The liveweight of the cattle ranged from 180 kg to 550 kg and in each field trial the administration of the MTEB was compared with either an alternative supplement or no supplement or both.

Comparison was made of the changes seen in the blood parameters of trace element status. The three used in this series of trials were plasma copper concentration, whole blood glutathione peroxidase (GSHPx) activity and plasma Vitamin $B_{12}$ concentration. These gave an indication of the copper, selenium and cobalt status of the animals.

The levels taken as being indicative of adequate marginal and deficient status are those adopted by the Scottish Veterinary Investigation Service and are given below;

| | Copper | Selenium | Cobalt |
|---|---|---|---|
| Blood analysis | plasma copper | whole blood GSHPx activity | plasma Vitamin B12 |
| Units | umol/litre | iu/ml PCV | ng/litre |
| Adequate | 9.4 - 24 | >17 | >200 |
| Marginal | - | 8 - 17 | 150 - 200 |
| Deficient | <9.4 | <8 | <150 |

In three trials the liveweight gain of the animals was also recorded.

# SECTION 3

**Experiment 3.1.**
**Observation trials using the MTEB in growing cattle at grass on sites where combined trace element inadequacies had been experienced in the past.**

**Experiment 3.2.**
**A field trial with autumn calving beef cows to compare the use of the MTEB with parenteral trace element supplements.**

**Experiment 3.3.**
**A field trial with autumn calving beef suckler cows and growing calves given either the MTEB or no supplement.**

**Experiment 3.4.**
**The use of the MTEB in beef suckler cows and the effect on the selenium status of the cows and their calves.**

**Experiment 3.5**
**The use of the MTEB in winter housed calves given hay and concentrates and subsequently at grass.**

**Experiment 3.6.**
**The retention of the MTEB when administered to calves at grass and the effect on their selenium status.**

**Experiment 3.7.**
**The incorporation of a base weight into the MTEB to increase density and retention.**

**Experiment 3.8**
**The use of the MTEB in growing cattle on a site suspected to be deficient in selenium and cobalt.**

**Experiment 3.9**
**The use of the MTEB in yearling cattle grazing a known selenium and cobalt deficient site.**

**Experiment 3.10**
**Comparison of the blood responses of calves given either no supplement, the multiple trace element bolus (MTEB), the soluble glass bolus (SGB) or a MTEB containing double the normal quantities of cobalt and selenium (MTEB SC+).**

## Experiment 3.1.

## Observation trials using the MTEB in growing cattle at grass on sites where combined trace element inadequacies had been experienced in the past.

### Introduction

A series of six trials were conducted in conjunction with Glasgow University Veterinary Practice where, because of past experience, various forms of individual preventive supplements with individual trace elements had been advocated as a general routine. Giving the MTEB as a single treatment could eliminate this need for separate injections and bolus administrations.

### Materials and methods

At or close to the time of transfer of cattle to upland grazing in the spring, on six separate sites a random half of each set of animals were given either the MTEB or no supplement or the separate injections and other bolus supplements as detailed in Table 3.1.

The number of cattle in each group varied from 10 to 50 depending upon the site. At each site blood samples were obtained from at least five animals per group on the day of administration and subsequently at intervals ranging up to 160 days. Estimations of plasma copper and Vitamin $B_{12}$ concentrations and whole blood glutathione peroxidase (GSHPx) activity were determined by the local Veterinary Investigation Centre.

**Table 3.1 Animals and other trace element supplements given in parallel with the MTEB at each of six sites.**

| Site | Cattle | Other trace element supplements given | | |
|---|---|---|---|---|
| | | Copper injection* | Selenium injection* | Cobalt bullet*** |
| 1 | 1 year old Limousin cross | YES | NO | YES |
| 2 | 300 kg Friesian male castrates | NO | NO | NO |
| 3 | 400 kg Friesian male castrates | NO | NO | NO |
| 4 | 1 year old Charolais cross | NO | NO | NO |
| 5 | 250-400 kg mixed beef crosses | YES | YES | NO |
| 6 | 1 year old Limousin cross | YES | NO | YES |

* 5 ml Bovicoppa injection. Rycovet Ltd. 50 mg Cu per ml as copper calcium edatate

** 5 ml Deposel injection. Rycovet Ltd. 250 mg Se as barium selenate

*** 1 Permaco Cobalt bullet. Coopers Pitman Moore Ltd.

## Results

The mean values for the analyses of the bloods for the cattle at each of the six sites are shown in Table 3.2. Although each of the sites was initially expected to be deficient in one or more of the elements only one (Site 6) was deficient in copper, only three (Sites 3, 4 and 5) were deficient in selenium and four were either deficient or marginally deficient in cobalt. Due to a misunderstanding plasma copper concentrations were not determined for Site 3.

### Plasma copper

At sites 1 and 2 the plasma copper concentrations were within the adequate range both initially and throughout both experiments. The MTEB or a copper injection gave similar mean values. The absence of an unsupplemented group unfortunately means that it cannot be ascertained if either treatment improved values or prevented a decline. On site 5 the MTEB increased plasma levels from marginally deficient to adequate whereas the values of those given a copper injection fell from adequate to deficient. This occurred after 120 days and reflects the relatively short protective life of the injection. At sites 4 and 6 both the MTEB and the unsupplemented groups had increased plasma copper values over the season and therefore the effect of the MTEB could not be assessed.

### Whole blood (GSHPx)

At sites 1, 2 and 6 the initial whole blood GSHPx activities were very high. At site 6 they increased to in excess of 100 iu/ml PCV by day 160 for both MTEB and unsupplemented groups. This lack of response to the MTEB was also evident at site 1 where the values for both groups fell over days 0 to 60. However at site 2 the MTEB group showed only a small drop in GSHPx activity from 85 to 72 units whereas the unsupplemented group fell from 84 to 48 units/ml PCV.

At sites 3, 4 and 5 where the GSHPx activities were initially low the administration of the MTEB gave significant increases, and all supplemented animals were restored to adequate blood levels. At site 5 where the alternative supplement was a barium selenate injection the increase shown by the MTEB group 5 to 27 units was greater than shown by the injected group of 6 to 16 units.

## Plasma Vitamin $B_{12}$

The conventional supplement used in attempts to increase cobalt status in these trials was the cobalt bullet (Permaco). At sites 1 and 6 where the MTEB was compared to that supplement similar results were recorded. After 60 days at site 1 both groups were still classed as deficient with no significant change in the plasma Vitamin $B_{12}$ values. Site 6 showed a similar result at 60 days but there was an improvement by both supplemented groups to day 160 when values were within the adequate range.

At site 5 both the MTEB and unsupplemented groups showed increased mean values over 120 days. In contrast, mean values of both groups fell at sites 2 and 4 and at site 3 there was no significant change over days 0 to 80.

## Discussion

From these results it would seem clear that the administration of the MTEB can increase and maintain plasma copper levels equal to if not better than a single copper injection.

For selenium, where the initial status was low, improvements were significant and occurred within 40 days. At one site where the MTEB was compared to a barium selenate injection the response recorded was equal.

Examination of the plasma Vitamin $B_{12}$ results reveals very little in favour of the MTEB. All that can be said is that the conventional supplement, the cobalt bullet, gives a similar response.

**Table 3.2** **Mean plasma copper, whole blood glutathione peroxidase and plasma Vitamin $B_{12}$ concentrations for groups of cattle (minimum of five animals per group bled on each site) given either the MTEB, a single or combination of trace element supplements or no supplement.**

| | | Plasma copper umol/litre Copper | | | Whole blood GSHPx iu/ml PCV Selenium | | | Plasma Vitamin B12 ng/litre Cobalt | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Trial No** | **Days** | **MTEB** | **Inj** | **Nil** | **MTEB** | **Inj** | **Nil** | **MTEB** | **Bullet** | **Nil** |
| **1** | **0** | 13.9 | 13.4 | | 92 | 102 | | 164 | 144 | |
| | **60** | 12.6 | 13.5 | | 63 | 51 | | 107 | 135 | |
| **2** | **0** | 12.4 | | 12.6 | 85 | | 85 | 234 | | 346 |
| | **60** | 10.6 | | 12.5 | 71 | | 47 | 127 | | 98 |
| | **150** | 14.5 | | 14.6 | 72 | | 48 | 195 | | 176 |
| **3** | **0** | nd | | nd | 7 | | 2 | 62 | | 50 |
| | **40** | nd | | nd | 21 | | 3 | 68 | | 42 |
| | **80** | nd | | nd | 22 | | 2 | 87 | | 52 |
| **4** | **0** | 11.9 | | 9.8 | 16 | | 10 | 296 | | 252 |
| | **60** | 12.7 | | 11.5 | 45 | | 8 | 130 | | 173 |
| | **90** | 13.1 | | 12.4 | 52 | | 18 | 128 | | 198 |
| **5** | **0** | 9.3 | 13.4 | | 5 | 6 | | 170 | 78 | |
| | **120** | 13.7 | 8.0 | | 27 | 16 | | 247 | 240 | |
| **6** | **0** | 6.5 | 6.1 | | 62 | 63 | | 119 | 123 | |
| | **60** | 15.0 | 11.0 | | 100 | 102 | | 140 | 122 | |
| | **160** | 14.0 | 13.5 | | 110 | 102 | | 200 | 218 | |

nd - not determined

**Experiment 3.2.**

**A field trial with autumn calving beef cows to compare the use of the MTEB with parenteral trace element supplements.**

## Introduction

This experiment was carried out to evaluate the MTEB against parenteral therapy. The site was chosen as it had a history of copper, selenium and cobalt deficiency.

## Materials and methods

Thirty-one Hereford cross suckler cows of mixed ages were used in this experiment. The cows were autumn calving and during the period of the experiment (June-November) grazed predominantly upland pasture. There was a past history of trace element inadequacies which were generally controlled by copper, selenium and Vitamin $B_{12}$ injections.

The individually identified cows were divided into 3 groups and in June the following supplements were given.

(a) Seventeen cows received two MTEB.

(b) Seven cows were given:
5 ml Deposel injection (Rycovet Ltd) (250 mg selenium as barium selenate)
2 ml Bovicoppa injection (Rycovet Ltd) (100 mg copper as copper calcium edetate)
3 ml Multivet $B_{12}$ injection (C-Vet Ltd) (3000 mcg cyanocobalamin).

(c) Seven cows received no treatment.

The second group represented the parenteral supplements given to this class of cattle and had been used previously in this herd.

Blood samples were taken on the same day as the supplements were given and again after 78 and 162 days. Analyses were conducted by the local Veterinary Investigation Service Laboratory for plasma copper and plasma Vitamin $B_{12}$ concentrations and for whole blood glutathione peroxidase (GSHPx) activity.

## Results

The results of the analyses are given in Tables, 3.3, 3.4 and 3.5. Following the discovery of deficient levels of copper and GSHPx recorded for the unsupplemented group on day 78 each of that group were given two MTEB one week later.

## Plasma copper

The mean values given in Table 3.3 exclude those cows which had an initial concentration greater than 9.4 umol/litre (shown within boxes) as it was considered that these had either a reduced or no possibility of improvement as a result of the treatments.

The mean plasma copper concentration for all three treatments was initially 5.5 - 6.2 umol/litre. By day 78 this had increased to mean values of 12.9 and 13.0 umol/litre as a result of giving boluses or injections. In contrast the mean value for the group receiving no supplement was only 9.3 umol/litre and two out of four animals under consideration had values which were still very low. In consequence they were given two MTEB on day 85 and by day 162 their mean concentration had increased to 15.7 umol/litre which was comparable to the initially bolused group mean of 15.7 umol/litre and the injected group mean of 14.6 umol/litre. Unfortunately no sample was obtained on day 162 from four of the original 17 cattle given the MTEB, but with these exceptions no bolused or injected animal had a blood copper concentration below 9.4 umol/litre.

It is concluded that over the experimental period of 162 days administration of the MTEB was as effective as the copper injection in increasing initially low blood copper concentrations.

**Table 3.3** (contd) Statistical Evaluation

Because the original No Supplement group of cattle were given two boluses on day 85 the data can only be evaluated at day 78.

| | Mean difference | SED | Significance |
|---|---|---|---|
| | Day 0 - Day 78 | | |
| Bolus | + 7.4 | 0.50 | <0.001 |
| Injection | + 6.8 | 1.61 | <0.01 |
| No Supplement | + 2.7 | 2.10 | NS |

| | Mean difference | SED | Significance |
|---|---|---|---|
| Bolus v No Supplement | 3.6 | 1.53 | <0.05 |
| Injection v No Supplement | 3.7 | 2.05 | NS |

**Table 3.3** **Plasma copper concentrations (umol/litre) at days 0, 78 and 162 for cows given the MTEB, a copper calcium edatate injection or no supplement.**

| BOLUSED | | | INJECTION | | | NO SUPPLEMENT* | | |
|---|---|---|---|---|---|---|---|---|
| DAYS | | | | | | | | |
| **0** | **78** | **162** | **0** | **78** | **162** | **0** | **78** | **162** |
| 5.8 | 11.2 | 15.7 | 7.7 | 10.4 | 17.3 | 4.1 | 4.9 | 12.6 |
| 6.4 | 12.8 | 12.6 | 7.4 | 11.2 | 12.6 | 6.6 | 10.0 | 15.7 |
| 4.4 | 12.2 | 15.7 | 4.5 | 18.7 | 15.0 | 6.4 | 14.5 | 15.7 |
| 5.6 | 13.3 | 17.3 | 5.1 | 11.6 | 12.6 | 5.3 | 7.8 | 18.8 |
| 8.2 | 11.4 | 14.4 | 6.1 | 13.3 | 15.7 | | | |
| 4.9 | 14.9 | 18.8 | | | | | | |
| 4.3 | 10.8 | n.s. | 12.3 | 13.1 | n.s. | 10.4 | 16.3 | n.s. |
| 6.7 | 13.9 | n.s. | 10.6 | 12.9 | n.s. | 10.9 | 17.7 | n.s. |
| 3.9 | 12.6 | 18.8 | | | | 11.1 | 13.9 | 22.0 |
| 3.9 | 13.7 | 15.7 | | | | | | |
| 5.4 | 14.3 | 15.7 | | | | | | |
| 5.6 | 14.7 | 14.1 | | | | | | |
| 5.9 | 12.0 | 14.1 | | | | | | |
| 10.8 | 14.5 | 15.7 | | | | | | |
| 12.0 | 15.5 | 18.8 | | | | | | |
| 10.4 | 14.7 | n.s. | | | | | | |
| 10.2 | 12.0 | n.s. | | | | | | |
| **5.5** | **12.9** | **15.7** | **6.2** | **13.0** | **14.6** | **5.6** | **9.3** | **15.7** |
| 1.22 | 1.35 | 1.98 | 1.40 | 3.34 | 2.04 | 1.15 | 4.05 | 2.53 |
| 13 | 13 | 11 | 5 | 5 | 5 | 4 | 4 | 4 |

**mean**
**s.d.**
**n**

n.s. no sample

* MTEB given to no supplement group on day 85.

**Whole blood GSHPx**

The mean whole blood GSHPx activity of all 31 cows at day 0 was 35 iu/ml PCV (s.d. 12.2) and only three of these could be classed as even marginally deficient. The selenium status of the herd as a whole therefore appeared to be adequate.

For the cows receiving no supplement there was a significant fall ($P<0.01$) over days 0-78 from 30 to 16 iu/ml PCV. These low levels caused concern and as was stated earlier on day 85 these animals were given two MTEB which resulted in a significant increase ($P<0.05$) in GSHPx activities by day 162 (16 to 37 iu/ml PCV).

The use of the MTEB significantly ($P<0.01$) increased the GSHPx activities from days 0 to 78 and between days 78 and 162 these increased values were maintained. Three animals were marginal or deficient at day 0. By days 78 and 162 these were reduced to nil and one respectively.

The barium selenate injected group showed no significant increase over days 0 to 78 but over days 78 to 162 a significant increase in GSHPx activities was recorded. No animals showed marginal/deficient levels at day 0 or at subsequent samplings. The overall increases in GSHPx values from the MTEB and the barium selenate injection of 8 and 14 iu/ml PCV respectively were not significantly different.

**Table 3.4** (contd) Statistical Evaluation

Because the original No Supplement group of cattle were given two boluses on day 85 the data can only be evaluated at day 78.

| | Mean difference | SED | Significance |
|---|---|---|---|
| | Day 0 - Day 78 | | |
| Bolus | 9 | 4.9 | NS |
| Injection | - 3 | 5.2 | NS |
| No Supplement | - 14 | 3.7 | <0.01 |

| | Mean difference | SED | Significance |
|---|---|---|---|
| Bolus v No Supplement | 28 | 4.2 | <0.001 |
| Injection v No Supplement | 20 | 3.9 | <0.001 |

**Table 3.4** **Whole blood glutathione peroxidase activities (iu/ml PCV) at days 0, 78 and 162 for cows given the MTEB, a barium selenate injection or no supplement.**

| | BOLUSED | | | INJECTION | | | NO SUPPLEMENT* | | |
|---|---|---|---|---|---|---|---|---|---|
| **DAYS** | **0** | **78** | **162** | **0** | **78** | **162** | **0** | **78** | **162** |
| | 15 | 35 | 36 | 36 | 27 | 40 | 43 | 24 | 56 |
| | 50 | 42 | 42 | 40 | 45 | 79 | 37 | 19 | 20 |
| | 28 | 48 | 41 | 29 | 25 | 48 | 22 | 7 | 21 |
| | 21 | 37 | 44 | 38 | 41 | 54 | 26 | 14 | 44 |
| | 48 | 68 | 62 | 28 | 30 | 45 | 19 | 17 | n.s. |
| | 38 | 38 | 50 | 47 | 34 | n.s. | 28 | 15 | n.s. |
| | 44 | 59 | n.s. | 58 | 47 | n.s. | 32 | 19 | 45 |
| | 33 | 22 | n.s. | | | | | | |
| | 31 | 33 | 27 | | | | | | |
| | 16 | 24 | 23 | | | | | | |
| | 17 | 37 | 18 | | | | | | |
| | 29 | 50 | 33 | | | | | | |
| | 50 | 38 | 49 | | | | | | |
| | 43 | 47 | 50 | | | | | | |
| | 26 | 26 | 37 | | | | | | |
| | 51 | 63 | n.s. | | | | | | |
| | 58 | 73 | 90 | | | | | | |
| **mean** | **35** | **44** | **43** | **39** | **36** | **53** | **30** | **16** | **37** |
| **s.d.** | 13.7 | 15.1 | 17.9 | 10.5 | 8.8 | 15.3 | 8.4 | 5.3 | 16.0 |
| **n** | 17 | 17 | 14 | 7 | 7 | 5 | 7 | 7 | 5 |

n.s. no sample

* MTEB given to no supplement group on day 85.

## Plasma Vitamin $B_{12}$

The mean plasma Vitamin $B_{12}$ concentration of all the 31 cows on day 0 was 91 ng/litre (s.d. 38.9). Concentrations below 150 ng/litre are classed as deficient, and 29 of the cows were in that category with one marginally deficient and only one adequate.

By day 78 neither the administration of Vitamin $B_{12}$ injection nor the MTEB had altered the mean plasma Vitamin $B_{12}$ concentration by any significant amount. The unsupplemented control group had also not shown a change in values. Over days 78-162 both the group initially given MTEB and the unsupplemented group given MTEB on day 85 showed no significant change in values. The Vitamin $B_{12}$ injected group over that period however showed a significant increase ($P < 0.05$) from 93 to 209 ng/l Vitamin $B_{12}$. This is difficult to explain since a single Vitamin $B_{12}$ injection was given on day 0 would be hardly likely to show an increase at day 162 when there was no improvement shown on day 78.

## Discussion

The use of the MTEB on this site gave comparable results to injections of both copper calcium edetate and barium selenate as measured by copper and selenium blood status. This was in a situation where other cattle receiving no supplement remained at an inadequate status in both respects.

No response in plasma Vitamin $B_{12}$ was recorded for either the administration of the MTEB or the Vitamin $B_{12}$ injection.

**Table 3.5** (contd) Statistical Evaluation

Because the original No Supplement group of cattle were given two boluses on day 85 the data can only be evaluated at day 78.

| | Mean difference | SED | Significance |
|---|---|---|---|
| | Day 0 - Day 78 | | |
| Bolus | + 23 | 26.5 | NS |
| Injection | + 19 | 48.2 | NS |
| No Supplement | + 16 | 50.4 | NS |

| | Mean difference | SED | Significance |
|---|---|---|---|
| Bolus v No Supplement | + 34 | 23.0 | NS |
| Injection v No Supplement | 0 | 30.0 | NS |

**Table 3.5** **Plasma Vitamin $B_{12}$ concentrations (ng/litre) at days 0, 78 and 162 for cows given the MTEB, a Vitamin $B_{12}$ injection or no supplement.**

| | BOLUSED | | | INJECTION | | | NO SUPPLEMENT* | | |
|---|---|---|---|---|---|---|---|---|---|
| **DAYS** | **0** | **78** | **162** | **0** | **78** | **162** | **0** | **78** | **162** |
| | 75 | 350 | 127 | 60 | 50 | 145 | 90 | 65 | 136 |
| | 105 | 75 | 192 | 50 | 50 | 165 | 75 | 60 | 74 |
| | 235 | 65 | 203 | 55 | 250 | 319 | 55 | 80 | 137 |
| | 100 | 100 | 192 | 50 | 115 | 165 | 135 | 100 | 371 |
| | 75 | 75 | 148 | 95 | 65 | 253 | 60 | 160 | n.s. |
| | 100 | 140 | 376 | 90 | 50 | n.s. | 70 | 80 | n.s. |
| | 170 | 140 | n.s. | 115 | 70 | n.s. | 55 | 105 | 179 |
| | 130 | 180 | n.s. | | | | | | |
| | 70 | 250 | 189 | | | | | | |
| | 45 | 50 | 74 | | | | | | |
| | 90 | 65 | 153 | | | | | | |
| | 75 | 195 | 150 | | | | | | |
| | 90 | 50 | 206 | | | | | | |
| | 105 | 130 | 192 | | | | | | |
| | 105 | 95 | n.s. | | | | | | |
| | 90 | 140 | 146 | | | | | | |
| **mean** | **104** | **127** | **182** | **74** | **93** | **209** | **77** | **93** | **179** |
| **s.d.** | 43.2 | 78.9 | 68.9 | 26.1 | 73.0 | 74.2 | 28.4 | 33.9 | 113.5 |
| **n** | 17 | 17 | 14 | 7 | 7 | 5 | 7 | 7 | 5 |

n.s. no sample

* MTEB given to no supplement group on day 85.

## Experiment 3.3.

## A field trial with autumn calving beef suckler cows and growing calves given either the MTEB or no supplement.

### Introduction

This experiment was carried out on a known selenium deficient site. A beef suckler herd and the previous years calves from the herd were used to test the MTEB under circumstances of moderate to severe selenium deficiency.

### Materials and method

#### Suckler cows

Twenty autumn calving Blue-grey beef suckler cows of mixed ages were housed in January and were given silage plus concentrate with an added mineral mix. At housing all the cows were given one Copporal capsule (RMB Animal Health Ltd) which contains 8.8 g of copper as copper oxide needles. They were transferred to grass in May.

In August some six weeks pre-calving ten of the cows were selected at random and given the MTEB and the other ten cows were unsupplemented. The cows remained at grass until the end of the experiment. Blood samples were taken in August and 120 days later. Analyses for plasma copper and plasma Vitamin $B_{12}$ concentrations and for whole blood glutathione peroxidase (GSHPx) activities were undertaken by the local Veterinary Investigation Service.

#### Growing cattle

Twenty Charolais cross heifer calves aged 9-11 months and weighing approximately 325-350 kg were used in this experiment. These were the calves born in the previous year to the Blue-grey cows described above. They were at grass for the whole of the experimental period.

In August ten calves were selected at random and given two MTEB and the other ten were given no supplement. Blood samples were taken in August and 120 days later and were analysed for plasma copper and plasma Vitamin $B_{12}$ concentrations, and for whole blood GSHPx activities.

## Results

### Suckler cows

The initial mean plasma copper, whole blood GSHPx and plasma Vitamin B12 values for all twenty cows were 13.1 umol/litre (s.d. 3.3), 6 iu/ml PCV (s.d. 3.1) and 148 ng/litre (s.d. 59.4) respectively (Table 3.6). These blood values indicated that the herd was of adequate copper status (although two cows were at or below 9.4 umol/l) but were particularly deficient in respect of selenium and also deficient in cobalt.

Both of the cows with initially low plasma copper concentrations were in the group receiving no supplement and both were of apparently adequate normal status by day 120. Otherwise the initial plasma copper concentrations were too high to allow a response to the MTEB to be apparent.

The mean GSHPx activities for both the cows receiving no supplement and those given the MTEB increased from day 0 to day 120. For those given the MTEB the increase (4 to 36 iu/ml PCV) was however significantly greater ($P<0.01$) than for the no supplement group (7 to 15 u/ml PCV). Additionally by day 120 only one bolused animal remained below the adequate range. In contrast seven of the ten cows receiving no supplement remained below the adequate range.

The plasma Vitamin B12 concentrations for both groups significantly increased ($P<0.01$) over the 120 days and there was no significant difference between the increases shown for the cows given the MTEB and for those given no supplement.

**Table 3.6** **The plasma copper, whole blood glutathione peroxidase and plasma Vitamin B12 values of suckler cows given either the MTEB or no supplement.**

| | Plasma copper umol/l | | Whole blood GSHPx iu/ml PCV | | Plasma Vitamin $B_{12}$ ng/l | |
|---|---|---|---|---|---|---|
| **DAYS** | **0** | **120** | **0** | **120** | **0** | **120** |
| BOLUSED | | | | | | |
| | 14.9 | 18.1 | 5 | 41 | 127 | 188 |
| | 15.7 | 17.7 | 4 | 47 | 105 | 229 |
| | 13.3 | 16.5 | 6 | 11 | 137 | 211 |
| | 11.8 | 20.4 | 4 | 54 | 80 | 173 |
| | 12.6 | 17.3 | 3 | 28 | 153 | 244 |
| | 11.0 | 15.7 | 4 | 32 | 326 | 278 |
| | 21.9 | 20.4 | i.s. | i.s. | 143 | 208 |
| | 16.5 | 17.3 | 7 | 46 | 213 | 190 |
| | 14.1 | 18.8 | 3 | 44 | 137 | 187 |
| | 13.3 | 13.3 | 4 | 18 | 101 | 242 |
| **mean** | **14.7** | **17.4** | **4** | **36** | **152** | **215** |
| **s.d.** | 3.10 | 2.21 | 1.3 | 14.4 | 70.8 | 32.8 |
| **n** | 10 | 10 | 9 | 9 | 10 | 10 |
| **DAYS** | **0** | **120** | **0** | **120** | **0** | **120** |
| NO SUPPLEMENT | | | | | | |
| | 14.9 | 17.3 | 3 | 12 | 186 | 252 |
| | 15.7 | 16.5 | 5 | 12 | 200 | 269 |
| | 12.6 | 14.2 | 10 | 17 | 170 | 300 |
| | 11.0 | 19.6 | 14 | 38 | 103 | 117 |
| | 9.4 | 16.5 | 8 | 3 | 136 | 213 |
| | i.s. | 14.9 | 6 | 15 | 229 | 399 |
| | 5.5 | 19.6 | 2 | 4 | 78 | 216 |
| | 11.8 | 16.5 | 4 | 10 | 108 | 173 |
| | 11.0 | 18.8 | 11 | 33 | 118 | 222 |
| | 12.6 | 12.6 | 8 | 14 | 117 | 177 |
| **mean** | **11.6** | **16.7** | **7** | **16** | **145** | **234** |
| **s.d.** | 3.01 | 2.31 | 3.8 | 11.4 | 49.0 | 78.0 |
| **n** | 9 | 10 | 10 | 10 | 10 | 10 |
| **SED** | 1.36 | 1.01 | 1.27 | 6.00 | 27.2 | 50.0 |
| **Sig** | NS | NS | NS | $P<0.01$ | NS | NS |

i.s. - insufficient sample.

**Growing cattle**

The initial mean plasma copper, whole blood GSHPx and plasma Vitamin $B_{12}$ values for all twenty calves were 10.9 umol/litre (s.d. 3.86), 12 iu/ml PCV (s.d. 5.6) and 197 ng/litre (s.d. 47.8) respectively (Table 3.7) showing a similar situation of selenium and cobalt deficiency to that of the cows. Seven of the calves had initial plasma copper values below 9.4 umol/litre.

By day 120 all seven calves had adequate plasma copper concentrations, the increases for these and all the other calves were as large for the animals given no supplement as for those given the MTEB. This may have resulted from a general response to supplementary concentrate feeding during the last months of the experiment. No conclusion could therefore be made regarding the effectiveness of the copper provided by the boluses.

The administration of the MTEB significantly ($P<0.01$) increased the GSHPx activities of the calves from 12 to 42 iu/ml PCV. In contrast the unsupplemented group showed no significant change in values from 12 to 11 iu/ml PCV. As with the cows only one bolused animal remained below the adequate range at day 120 but for the calves receiving no supplement eight of the ten remained in the deficient category.

Both groups showed no significant change in plasma Vitamin $B_{12}$ levels over the period of the experiment.

**Conclusion**

These experiments showed that administration of the MTEB significantly increased the selenium status of both adult and growing cattle and if the initial status as measured by whole blood GSHPx activities is even extremely deficient that the supply of selenium from the MTEB is sufficient to increase GSHPx activities to adequate levels for a period of at least 120 days.

As in earlier experiments no improvement in cobalt status as measured by plasma Vitamin $B_{12}$ concentrations was observed from administration of the MTEB.

**Table 3.7** **The mean plasma copper, whole blood glutathione peroxidase and plasma Vitamin $B_{12}$ values of growing cattle given either the MTEB or no supplement.**

| | Plasma copper umol/l | | Whole blood GSHPx iu/ml PCV | | Plasma Vitamin B12 ng/l | |
|---|---|---|---|---|---|---|
| **DAYS** | **0** | **120** | **0** | **120** | **0** | **120** |
| BOLUSED | | | | | | |
| | 6.3 | 25.2 | 13 | 26 | 190 | 270 |
| | 10.2 | 16.5 | 6 | 12 | 238 | 191 |
| | 14.1 | 19.6 | 11 | 34 | 312 | 233 |
| | 7.1 | 16.5 | 5 | 50 | 227 | 181 |
| | 14.9 | 26.7 | 19 | 34 | 188 | 226 |
| | 13.3 | 18.1 | 14 | 59 | 192 | 104 |
| | 11.0 | 18.8 | 11 | 50 | 143 | 134 |
| | 7.9 | 20.4 | 17 | 56 | 265 | 219 |
| | 10.2 | 17.3 | 8 | 61 | 215 | 134 |
| | 7.1 | 20.4 | 12 | 31 | 232 | 182 |
| **mean** | **10.2** | **20.0** | **12** | **42** | **220** | **187** |
| **s.d.** | 3.11 | 3.48 | 4.5 | 16.5 | 46.7 | 51.8 |
| **n** | 10 | 10 | 10 | 10 | 10 | 10 |
| **DAYS** | **0** | **120** | **0** | **120** | **0** | **120** |
| NO SUPPLEMENT | | | | | | |
| | 3.9 | 23.6 | 8 | 3 | 154 | 250 |
| | 10.2 | 25.9 | 10 | 9 | 148 | 123 |
| | 6.3 | 14.1 | 6 | 15 | 201 | 161 |
| | 14.9 | 18.1 | 20 | 9 | 164 | 141 |
| | 7.9 | 20.4 | 5 | 12 | 134 | 181 |
| | i.s. | 21.9 | 11 | 9 | 184 | 145 |
| | 14.1 | 20.4 | 27 | 17 | 247 | 96 |
| | 14.9 | 18.8 | 11 | 15 | 169 | 211 |
| | 15.7 | 16.5 | 15 | 12 | 207 | 187 |
| | 16.5 | 18.1 | 11 | 7 | 122 | 105 |
| **mean** | **11.6** | **19.8** | **12** | **11** | **173** | **160** |
| **s.d.** | 4.64 | 3.45 | 6.7 | 4.2 | 37.6 | 48.2 |
| **n** | 10 | 10 | 10 | 10 | 10 | 10 |
| **SED** | 1.77 | 1.54 | 2.55 | 5.38 | 18.8 | 22.3 |
| **Sig** | NS | NS | NS | $P<0.001$ | NS | NS |

i.s. insufficient sample

## Experiment 3.4

## The use of the MTEB in beef suckler cows and the effect on the selenium status of the cows and their calves.

### Introduction

Selenium supplementation of the cow prepartum can enhance the status of the calf due to placental transfer of that element. (Kincaid & Hodgson, 1989).

Similarly, post partum supplementation may increase the level of selenium in the dams' milk further enhancing the status of the calf. (Hidiroglou et al., 1985).

Both methods are effective in providing an adequate status until weaning and this may thus avoid the need to supplement the calf directly. Since it is the young ruminant which is more prone to the manifestations of selenium deficiency this is a useful strategy to employ. This experiment examines the use of the MTEB in supplying selenium in this way.

### Materials and methods

Thirty-five spring calving Hereford cross Friesian cows in calf to a Charolais bull were used in the experiment. They were housed from November and fed 6 kg hay and 2 kg concentrate per day. The selenium contents of the feedstuffs are given in Table 3.8.

In mid-January the cows were weighed (mean 474 kg) and body condition scored (mean 2.75). The cows were then divided into two comparable groups, one with 23 cows and one with 12 cows based on age, liveweight, body condition score and anticipated calving date.

The larger group was given two MTEB on 27 January and the other group received no supplement. On that day the cows were blood sampled and this continued at intervals throughout the experiment. The blood samples were assayed for whole blood glutathione peroxidase (GSHPx) activity as an indicator of the selenium status of the animals.

Calving took place from March to early April. Calves were blood sampled at birth and at 3 and 6 weeks of age, thereafter they were sampled on the same days as the cows. The cows and their calves were transferred to grass in early May and they grazed the same pasture until the calves were weaned on 31 October. Herbage samples were taken throughout the grazing period and the mean selenium content is given in Table 3.8.

**Table 3.8 Selenium content (mg/kg DM) of hay, concentrate and herbage in Experiment 3.4.**

| | | Selenium content | % of dietary allowance (MAFF et al. 1983) |
|---|---|---|---|
| Hay | | 0.020 | 20 |
| Concentrate | | 0.027 | 27 |
| Herbage | May | 0.055 | 55 |
| | July | 0.044 | 44 |
| | September | 0.049 | 49 |

**Results**

The winter diet provided only 0.22 mg selenium/kg DM and the herbage 0.44-0.55 mg/kg DM. Both are considerably less than the recommended dietary allowance of 0.1 mg/kg/day (MAFF et al., 1983).

The mean whole blood GSHPx activities for cows and calves over the period of the trial are given in Table 3.9 and the GSHPx activities at calving and 3 and 6 weeks later are given in Table 3.10.

The mean initial GSHPx activity for all 35 cows was 27 iu/ml PCV. This was within the adequate range. The administration of the MTEB increased the mean GSHPx activity of that group after two months to 63 iu/ml PCV whereas the group receiving no supplement had a mean of 30 iu/ml PCV. Since the majority of cows calved in March this difference was also shown at calving (Table 3.10) with both the cows and their calves from the bolused group having significantly higher GSHPx activities at calving and the values for the calves being significantly greater at 3 and 6 weeks of age. It seems reasonable to assume that this was a result of placental transfer in combination with increased milk selenium concentrations.

**Table 3.9** **Mean whole blood glutathione peroxidase activities (iu/ml PCV) of bolused and unsupplemented cows and their calves over 288 days.**

| | COWS | | | | | CALVES | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Bolused** | | **No supplement.** | | | **Bolused** | | **No supplement** | | |
| **Days** | mean | s.d. | mean | s.d. | | mean | s.d. | mean | s.d. | |
| **0** | 26 | 5.0 | 30 | 6.5 | n.s. | - | - | - | - | |
| **61** | 63 | 13.3 | 30 | 14.6 | *** | - | - | - | - | |
| **132** | 65 | 24.0 | 26 | 16.4 | *** | 44 | 13.0 | 28 | 4.4 | ** |
| **167** | 52 | 18.0 | 18 | 5.2 | *** | 41 | 12.1 | 23 | 3.6 | *** |
| **204** | 37 | 16.0 | 17 | 6.9 | *** | 25 | 7.0 | 15 | 3.1 | ** |
| **231** | 34 | 12.2 | 18 | 5.1 | *** | 23 | 5.8 | 19 | 2.3 | n.s. |
| **288** | 20 | 8.7 | 17 | 5.4 | n.s. | 23 | 7.0 | 17 | 2.4 | ** |

Level of significance

P<0.001 ***
P<0.01 **
P<0.05 *

n.s. not significant

**Table 3.10 Mean whole blood glutathione peroxidase activities (iu/ml PCV) of bolused and unsupplemented cows and their calves at calving and the mean values for the calves at three and six weeks of age.**

| | | Bolused n=23 | | No supplement n=12 | | SED | Sig |
|---|---|---|---|---|---|---|---|
| | | mean | s.d. | mean | s.d. | | |
| **Calving** | cows | 63 | 18.4 | 27 | 11.1 | 5.0 | P<0.001 |
| | calves | 58 | 18.3 | 30 | 9.1 | 4.6 | P<0.001 |
| **Calves** | 3 weeks | 72 | 13.2 | 35 | 11.3 | 4.3 | P<0.001 |
| | 6 weeks | 65 | 17.3 | 37 | 16.6 | 6.0 | P<0.001 |

The MTEB supplemented group had significantly greater mean GSHPx activities in the cows until day 231 and for their calves until weaning at day 288 (Table 3.9). On the last three sampling occasions the cows and calves in the group receiving no supplement had GSHPx activities indicative of marginal selenium deficiency.

## Conclusion

The administration of the MTEB prepartum to these beef cows increased their GSHPx activities within two months and maintained a signficantly higher level than those cows given no supplement to 231 days after administration. Calves from the MTEB supplemented dams had significantly higher GSHPx activities at birth and the difference over the non-supplemented group was maintained until weaning.

The MTEB is therefore a useful means of providing selenium prepartum to the dam to enable the status of the calf to be improved at birth and to maintain adequate GSHPx levels until weaning.

## Experiment 3.5.

## The use of the MTEB in winter housed calves given hay and concentrates and subsequently at grass.

### Introduction

As described in Section 2 the main use of the MTEB was envisaged to be the supplementation of cattle at grass. It could however be used in a winter feeding situation as a replacement for a trace element mineral mix added to the concentrate. Equally where the diet consisted only of conserved forage it could be perhaps used as a novel supplement.

This experiment examines the effect of administering two MTEB to recently weaned beef suckler calves of about 250 kg liveweight.

### Materials and methods

The animals used in this experiment were fifteen Charolais cross heifers and steers which had been weaned two weeks prior to the start of the experiment and were aged approximately 7 months. They were allocated by sex and liveweight to form two balanced groups (mean initial liveweight 265 kg). The first group with eight animals were given the MTEB at housing. The other group received no supplement.

All the animals were kept together as one group in a covered building and straw was used as bedding. They were group-fed 2.5 kg of concentrate and 4 kg hay per head daily throughout the housing period.

The animals were weighed and blood sampled at regular intervals until transfer to grass in the spring. Following the period at grass the animals were weighed and blood sampled again at approximately 18 months of age before being housed for their second winter.

The blood samples were assayed for plasma copper and plasma Vitamin $B_{12}$ concentrations and for whole blood glutathione peroxidase (GSHPx) activities, as indicators of the copper, cobalt and selenium status of the calves.

**Results**

The liveweight changes of the calves over the experiment are shown in Table 3.11. The mean initial liveweight was 259 kg for the bolused groups and 271 kg for the unsupplemented group. The liveweight gain over the winter housing period was similar for both groups at 0.25 kg/day. The liveweight gain over the grazing period was greater for the bolused group 0.73 kg v 0.58 kg/day however this difference was not significant.

**Table 3.11 The liveweight (LW) and liveweight gain (LWG) of beef cross calves over the winter housing period and the subsequent grazing season.**

| | BOLUSED | | NO SUPPLEMENT | |
|---|---|---|---|---|
| | mean | s.d. | mean | s.d. |
| LW at housing | 259 | 35.4 | 271 | 37.6 |
| LW at transfer to grass | 297 | 35.9 | 311 | 47.7 |
| LWG (kg/day) over 154 day winter period | 0.25 | 0.062 | 0.26 | 0.121 |
| LW at housing for second winter (kg) | 420* | 36.5 | 410* | 34.1 |
| LWG (kg/day) over 184 day grazing period | 0.73 | 0.095 | 0.58 | 0.141 |

* bolused group n=7
control group n=6

The results of the blood analyses are shown in Table 3.12.

The plasma copper concentrations for all calves were within the adequate range of values (9.4 - 24.0 umol/litre). Both bolused and control calves remained within these values throughout the experiment and there were no significant differences between groups.

The whole blood GSHPx activities at the start of the experiment were indicative of marginal selenium status. The mean GSHPx activities were 17 and 13 iu/ml PCV for control and bolused groups respectively. The selenium status of similar calves reared by the same beef suckler cows in the following year was similarly low (Experiment 3.4) due to the low pasture selenium concentration of 0.05 mg/kg (Dietary allowance 0.1 mg/kg, MAFF et al, 1983).

The control group showed a significant increase in GSHPx activities over the winter period from 17 to 34 iu/ml PCV. However by the end of their second grazing period the GSHPx activities had fallen to a mean of 11 iu/ml PCV.

The bolused group also showed a significant ($P < 0.001$) increase over the winter period but this was from 13 to 85 iu/ml PCV. From day 42 and at all sampling occasions after housing the bolused group had signficantly greater GSHPx activities than the unsupplemented cattle. At the end of the second years grazing 338 days after the calves were bolused the mean GSHPx activity was still significantly greater than the unsupplemented group. The mean value of 85 iu/ml PCV at the end of the housing period of 154 days had however fallen to 23 units. With an expected bolus life of about 240 days, this was not unexpected.

The plasma Vitamin $B_{12}$ concentrations were above 200 ng/l for the majority of calves at the start of the experiment. Throughout the experiment the values were either marginal (150-200 ng/l) or adequate (over 200 ng/l) for both groups of calves. No significant difference between the groups was recorded.

The results from this experiment show that no response in plasma Vitamin $B_{12}$ is obtained by administering the MTEB. This is in broad agreement with all the field trials described within this thesis.

In summary, the selenium status of the calves was significantly improved by the administration of the MTEB prior to housing and the status was increased such that more than 300 days later the bolused calves still had a significantly greater mean GSHPx activity than those given no supplement and were within the adequate range. In contrast the majority of unsupplemented cattle were considered to be of deficient status.

**Table 3.12 Mean plasma copper, whole blood glutathione peroxidase and plasma Vitamin $B_{12}$ values for bolused and unsupplemented cattle over a period of 338 days.**

| | Plasma Copper u mol/litre | | | | | Whole blood GSHPx iu/ml PCV | | | | | | Plasma Vitamin $B_{12}$ ng/litre | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Days** | **Bolused mean** | **s.d.** | **Control mean** | **s.d.** | **Sig** | **Bolused mean** | **s.d.** | **Control mean** | **s.d.** | **SED** | **Sig P** | **Bolused mean** | **s.d.** | **Control mean** | **s.d.** | **Sig** |
| **0** | 15.7 | 2.47 | 15.4 | 1.84 | NS | 13 | 5.5 | 17 | 7.6 | 3.7 | NS | 209 | 36.8 | 248 | 55.2 | NS |
| **42** | 14.6 | 1.56 | 14.5 | 1.11 | NS | 27 | 10.8 | 16 | 3.0 | 4.3 | .05 | 198 | 52.6 | 203 | 62.2 | NS |
| **73** | 12.8 | 1.87 | 12.4 | 1.44 | NS | 76 | 43.8 | 19 | 2.0 | 16.5 | .001 | 147 | 43.8 | 189 | 69.0 | NS |
| **92** | 12.1 | 1.38 | 12.5 | 1.00 | NS | 87 | 9.6 | 27 | 8.0 | 4.8 | .001 | 185 | 41.7 | 226 | 48.5 | NS |
| **122** | 12.6 | 1.11 | 13.8 | 2.53 | NS | 87 | 19.7 | 33 | 4.8 | 7.7 | .001 | 170 | 45.8 | 196 | 84.4 | NS |
| **154** | 12.5 | 1.73 | 13.6 | 2.34 | NS | 85 | 8.6 | 34 | 2.2 | 3.4 | .001 | 186 | 41.2 | 190 | 67.4 | NS |
| **338** | 14.7 | 1.39 | 14.7 | 2.49 | NS | 23 | 4.2 | 11 | 4.2 | 2.3 | .001 | 258 | 123.6 | 158 | 13.3 | NS |

## Experiment 3.6

### The retention of the MTEB when administered to calves at grass and the improvement in their selenium status.

### Introduction

The initial investigational work reported in Sections 1 and 2 of this thesis was conducted with adult fistulated cows which were generally fed hay. Invariably both boluses were found in the reticulum and none were lost by regurgitation. It is however possible that a different situation may exist with younger cattle at grass. This experiment utilized the opportunity to bolus twenty six Friesian cattle of 180 kg liveweight which were at grass and were to be slaughtered as part of a separate joint experimental programme between the Departments of Veterinary Animal Husbandry and Veterinary Parasitology, the primary aim of which was to study the pathology of parasitic infection.

Routine blood sampling established that the cattle were of low selenium status as measured by whole blood glutathione peroxidase activity (GSHPx) and the opportunity was also taken to determine the effect of the MTEB in this respect.

### Materials and methods

Four weeks after transfer to grass, two MTEB were administered to each of twenty six Friesian and Holstein cross Friesian male cattle of mean liveweight 184 kg. The individual weight of each bolus and number of the calf which received them were recorded. Eight days subsequent to this a further four calves were brought onto the trial. These were not given boluses and with this limitation acted as an unsupplemented control group.

At the same time as bolus administration a blood sample was taken and assayed for whole blood GSHPx activity. Further blood samples were obtained at regular intervals throughout the trial which lasted until the slaughter of 13 of the cattle after 100 days.

## Results

### Whole blood GSHPx

The twenty six calves from which blood samples were obtained on day 0 showed a wide range of GSHPx activities (iu/ml PCV). These could however be split into two reasonably uniform groups. One group contained 16 calves all of which had GSHPx values below 30 units, (low Se status group; mean 17 iu/ml PCV, s.d. 7.9). Some of these had values indicative of deficiency. In the other group of ten calves the majority had values in excess of 60 units (high Se status group, mean 66 iu/ml PCV, s.d. 18.5). The range of values perhaps indicates that the calves originated from at least two different sources as might be expected for a batch of purchased calves of that type. Table 3.13 indicates that both bolused groups of calves showed significant increases in GSHPx activities these being from 17 units to 63 units and from 66 units to 83 units for the low status group and high status group respectively. In contrast the unsupplemented group showed no significant change in GSHPx values with a small fall from 80 units to 76 units.

The increase in values in the bolused group appeared to be related to the initial blood level, the low Se status group having a mean increase of 46 units and the high Se status group with a mean increase of 17 units. The difference between the two groups was significant ($P<0.01$).

**Table 3.13 The mean whole blood glutathione peroxidase activities (iu/ml PCV) of calves given the MTEB and those given no supplement.**

| | | | Bolused | | | |
|---|---|---|---|---|---|---|
| | No supplement | | Low Se status | | High Se Status | |
| No of calves | 4 | | 16 | | 10 | |
| **Days** | mean | s.d. | mean | s.d. | mean | s.d. |
| **0** | - | - | 17 | 7.9 | 66 | 18.5 |
| **14** | 80 | 16.2 | 29 | 9.4 | 65 | 19.7 |
| **42** | 81 | 18.4 | 48 | 12.1 | 68 | 16.5 |
| **63** | 76 | 17.6 | 53 | 15.1 | 70 | 12.4 |
| **84** | 76 | 12.6 | 63 | 13.3 | 83 | 19.8 |
| **mean change over days 0-84** | **-4a*** | 7.0 | **46b** | 12.2 | **17a** | 14.2 |

* mean change over days 14-84
a, b, Values with different subscripts are significantly different ($P<0.05$)

**Recovery of boluses**

Thirteen of the calves given the MTEB were slaughtered 100 days after the boluses were administered. No boluses were recovered from two of the calves and only one was recovered from each of two other calves. Table 3.14 gives the mean initial and final weights and Plate 11 shows two pairs of the recovered boluses. The bolus recovery rate of 77% was very disappointing, especially as no such loss of the MTEB had previously been recorded in fistulated cows.

In the light of this and other field observations the density of the 'All-trace' bolus was subsequently increased to 2.8 $g/cm^3$ to prevent regurgitation. The method by which this was done is described in Section 3.7.

The mean release rate over the 100 days of 0.45 g/bolus/day is similar to that recorded in fistulated cows of 0.43 g/bolus/day over 63 days in Experiment 2.11.

**Table 3.14 The initial and recovered weights and the release rate of the MTEB given to calves at grass and recovered at slaughter, 100 days after bolus administration.**

| | Initial bolus weight (g) | Final bolus weight (g) | Release rate g/bolus/day |
|---|---|---|---|
| **n** | 26 | 20 | 20 |
| **mean** | 83.6 | 38.8 | 0.45 |
| **s.d.** | 1.26 | 8.4 | 0.082 |

With a total mean release per day of 0.9 g matrix the two boluses supply 1.0 mg Se/day. The mean pasture selenium concentration was 0.06 mg/kg DM i.e. about 60% of the daily recommended allowance (MAFF et al, 1983). Assuming a DM intake of 5 kg for the calves the herbage would contribute 0.3 mg selenium/day and the animal would then receive 1.3 mg selenium in total per day. This is approximately two and a half times the recommended daily allowance for a 200 kg calf. Nevertheless this level of selenium intake did not lead to excessively high GSHPx activities and it did allow animals with a very low initial selenium status to regain adequate status in a fairly short period of time.

**Plate 11.**
**Pairs of boluses recovered from two calves 100 days after administration.**

**Experiment 3.7.**

**The incorporation of a base weight into the MTEB to increase density and retention.**

## Introduction

Observations from Experiments 3.4 and 3.6 and other reports from the field suggested that some regurgitation of the MTEB was occurring. This loss was largely independent of size of animal, diet and time from administration. Commercial sources estimated the level of regurgitation to be between 3 and 5% and it generally occurred when the bolus was more than half worn. Although this was a similar level to that shown by other bolus products, the MTEB had the lowest density at 2.6 g/cm$^3$ compared with 3.0 g/cm$^3$ for the pulse release anthelmintic bolus (Coopers Pitman Moore Ltd) and 3.3 g/cm$^3$ for the Morantel RDD (Elanco Products Ltd) and it was decided that it should be increased to 2.8 - 3.0 g/cm$^3$.

Attempts to increase the bolus density by altering the formulation were of limited success and altered the release rate drastically (Experiment 2.3). To avoid such problems it was decided to add a base weight to the existing formulation. As with the whole bolus this base weight was designed to be erodable and to leave no residue.

## Materials and methods

Iron powder was chosen to form the base weight as it was readily available at reasonable cost, would not damage the manufacturing machinery and when compressed would have a density of 5.2 g/cm$^3$. To enable the base weight to dissolve 5% of manganese sulphate was incorporated with the iron powder.

Twenty-six grammes of this mixture was compressed in a 19 mm mould to give a base weight 19 mm x 21 mm which was rounded at both ends.

The base weight was placed into the mould prior to the rest of the matrix and when compression occurred it was then incorporated into the bolus. Plate 12 shows the base weight and the position within the bolus.

To test that such a base weight would erode and leave no residue both the base weight alone and the bottom 2.5 cm of the resultant MTEB were cut off so that the base weight was exposed, these were placed in three rumen-fistulated cows.

The test period was 21 days and the cows were at grass for all of that period. The weight loss of the boluses was recorded as in Section 1.5.

**Results**

The inclusion of a base weight increased the bolus density to 2.84 g/cm$^3$ and the bolus weight increased to 106 g, i.e. 21 g base weight and 85 g of bolus matrix. Plate 13 shows the weighted bolus compared to the original type. The density increased as the bolus dissolved/eroded in the rumen. At 80 g it was 3.01 g/cm$^3$ and at 60 g 3.30 g/cm$^3$. Figure 3.1 demonstrates this relationship more clearly, the data being obtained by frequent measurements.

When only the base weight was given to the cows none were recovered after 7, 14 or 21 days. It seems reasonable to assume the base weight had thus disintegrated completely within 7 days.

Table 3.15 gives the mean loss in weight of the pairs of weighted boluses in fistulated cows. The loss in weight appeared to be rather more rapid than for the standard boluses. This may have been as a result of cutting the top portion of the bolus away after normal manufacture. In particular the rate of loss seemed to accelerate towards the virtual end of life of the bolus. In some cases parts of the end weights fractured and broke away and this would account for some of the loss in weight.

**Table 3.15 Weight loss (g) of the base portion of six boluses placed in pairs into three rumen-fistulated cows over a period of 21 days.**

| | **mean** | **s.d.** |
|---|---|---|
| Initial weight (g) | 40.2 | 0.33 |
| Weight loss (g) over days | | |
| 0-7 | 2.85 | 0.903 |
| 8-14 | 3.75 | 1.300 |
| 15-21 | 8.07 | 1.470 |
| 0-21 | 14.7 | 1.538 |
| Final wt. | 25.6* | 1.40 |

* The mean initial base weight was 26 g.

**Figure 3.1 The relationship between weight loss and density of a MTEB which incorporates a base weight.**

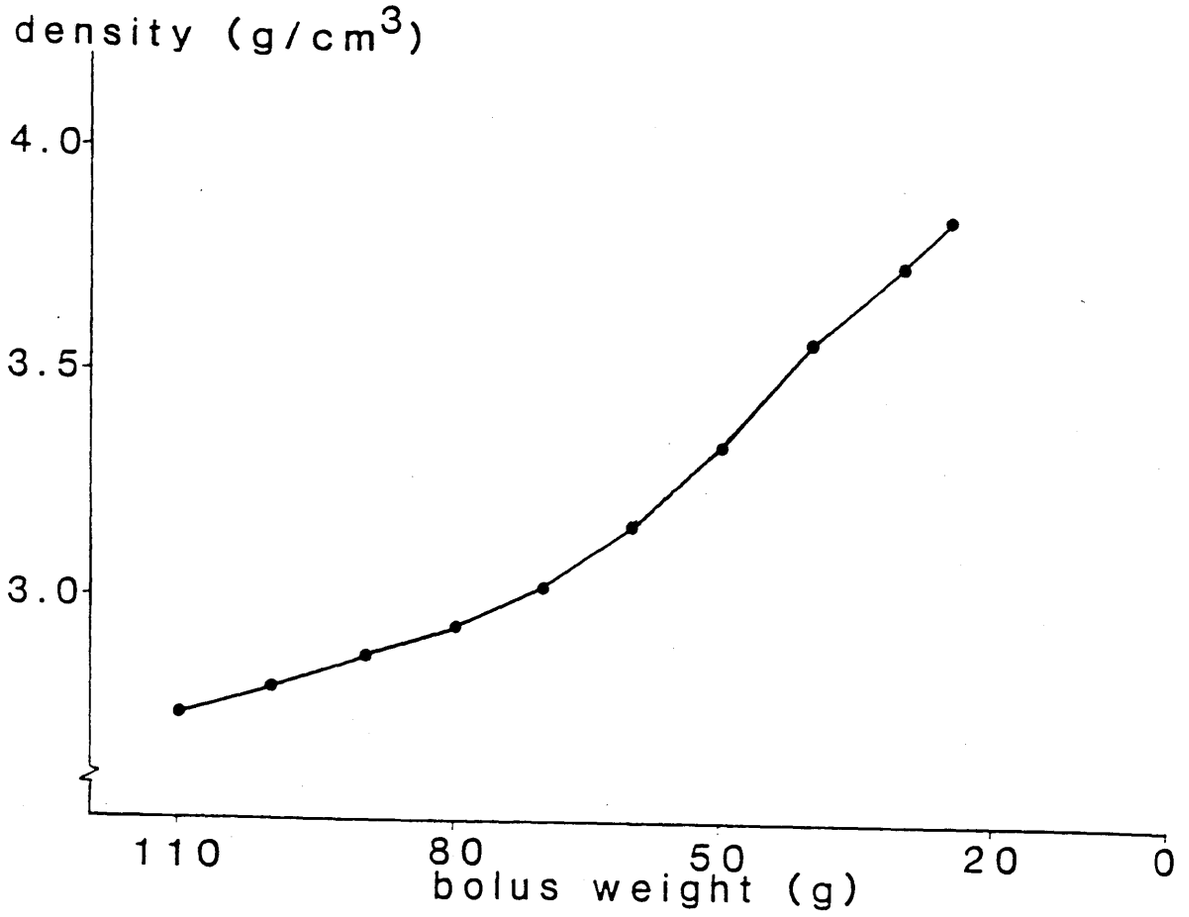
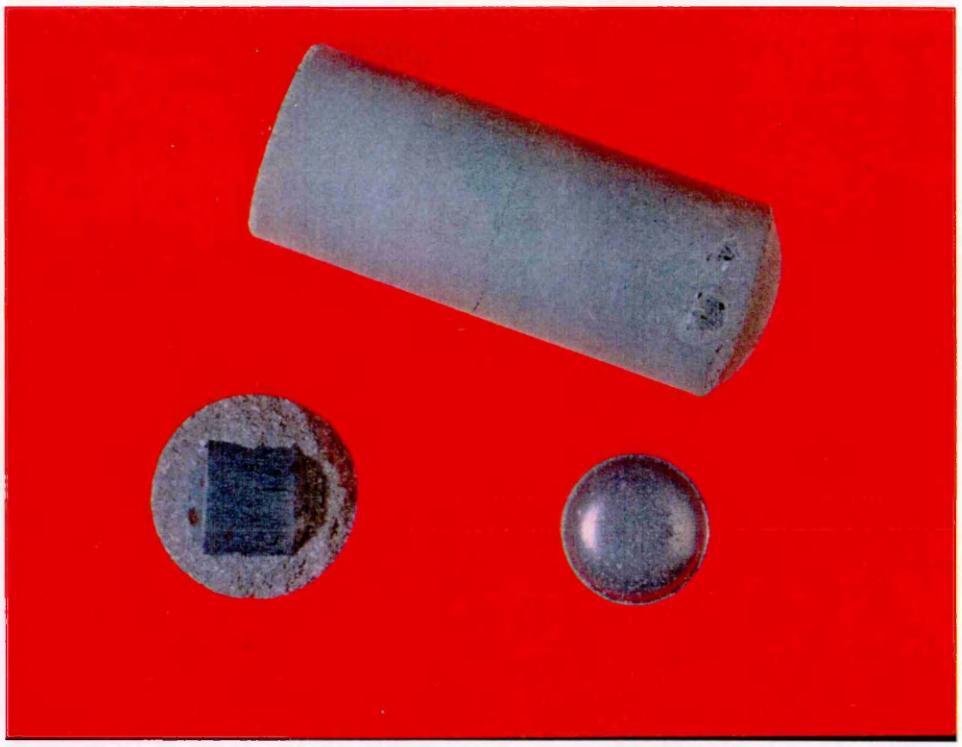

density (g/cm3)
4.0
3.5
3.0
110
80
50
20
0
bolus weight (g)

**Plate 12.**
**The position of the base weight at the bottom of the bolus and an uncoated bolus containing the base weight.**

**Plate 13.**
**An original MTEB and one which includes the erodable base weight, showing the increase in length due to the inclusion of the base weight.**



**Experiment 3.8.**

**The use of the MTEB in growing cattle on a site suspected to be deficient in selenium and cobalt.**

## Introduction

The cattle on this site had been found to be selenium and cobalt deficient as assessed by analyses of blood samples at the end of the previous grazing season but no clinical signs had been observed. The aim of this experiment was to determine if supplementation with the MTEB would restore normal blood concentrations.

## Materials and methods

One hundred and twenty Limousin and Blonde de Aquataine cross heifer and steer cattle aged approximately ten months were used in this experiment. They were divided into two equal groups of sixty calves. One group was given two MTEB three weeks after transfer to grass in the spring. The other group received no supplement. The MTEB given were those with the increased density. Blood samples were restricted to six animals in each group because of handling difficulties while the cattle were grazing. They were also weighed at intervals until the animals were sold. The blood samples were assayed for plasma copper and plasma Vitamin B12 concentrations and for whole blood glutathione peroxidase (GSHPx) activity. Herbage samples were taken at the start and the end of the trial in June and September respectively. The samples were taken from the fields which the cattle grazed for the whole of the 90 day period. They were obtained by walking across the field in diagonal sections and collecting herbage at intervals of 10 yards. The herbage thus obtained was then dried and milled prior to analysis.

## Results

The herbage analyses are shown in Table 3.16. For the sample taken in June the copper and cobalt contents were adequate but the selenium content was only 65% of the dietary allowance (MAFF et al., 1983). At the end of September there was a similar situation with the selenium content of the herbage at 0.04 mg/kg.

**Table 3.16 Trace element analyses of the herbage in Experiment 3.8 (mg/kg DM).**

| | Cobalt | Selenium | Copper | Sulphur | Molybdenum |
|---|---|---|---|---|---|
| June | 0.11 | 0.065 | 14 | 350 | 0.32 |
| September | 0.17 | 0.04 | 12 | 500 | 0.33 |

The overall mean liveweight at the start of the experiment for the 12 recorded animals was 370 kg (s.d. 26.0). The mean liveweight gains over the 90 day experimental period for the MTEB and non supplemented groups were 0.85 and 0.92 kg/day respectively with no significant difference between them. For all 12 animals the mean liveweight gain was 0.89 kg/day and the final liveweight 451 kg.

The individual concentrations of plasma copper, whole blood GSHPx activities and plasma Vitamin B12 concentrations for all the animals over the period of the experiment are given in Table 3.17.

For plasma copper all the animals were within the adequate range at the start of the trial. On day 60 however the plasma copper concentrations of three of the animals (two non supplemented and one given the MTEB) had fallen to marginal status. By the end of the trial all the animals were again within the adequate range of 9.4 to 24 umol/litre. No significant differences were recorded between the groups.

At the start of the trial the selenium status of both groups was high. The mean GSHPx activities for all twelve animals was 71 iu/ml PCV (s.d. 14.9). Sixty days after the boluses were given both groups showed a fall in GSHPx activity to 58 and 41 iu/ml PCV for those given the MTEB and for the non supplemented animals respectively.

By 90 days after the start of the trial the non supplemented group remained at about their sixty day level but those given the MTEB showed increased levels and at day 90 their GSHPx activities were greater than those shown on day 0. This gave a significantly greater ($P<0.01$) change in GSHPx activities over days 0-90 for the group given the MTEB over the non supplemented group (+8 v -21 iu/ml PCV). However even at day 90 all non supplemented cattle were of an adequate selenium status as measured by GSHPx activity.

The plasma Vitamin $B_{12}$ status was initially very low with a mean for all twelve animals of 85 ng/l (s.d. 20.4) where values of 200 ng/l or greater are considered to be adequate. The deficient status remained throughout the trial with no signficant differences between the two groups.

**Table 3.17 Mean plasma copper, whole blood glutathione peroxidase and Vitamin $B_{12}$ values for growing cattle given either the MTEB or no supplement.**

| | Copper umol/litre | | | GSHPx iu/ml PCV | | | | Vitamin $B_{12}$ ng/litre | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Days** | **0** | **60** | **90** | **0** | **60** | **90** | **0-90** | **0** | **60** | **90** |
| BOLUSED | | | | | | | Change | | | |
| | 13.5 | 10.7 | 11.8 | 81 | 53 | 91 | +10 | 95 | 50 | 80 |
| | 14.0 | 21.2 | 18.2 | 58 | 58 | 68 | +10 | 105 | 95 | 110 |
| | 15.4 | 8.1 | 16.3 | 60 | 39 | 63 | +3 | 115 | 125 | 140 |
| | 14.8 | 11.3 | 15.7 | 82 | 63 | 89 | +7 | 95 | 85 | 95 |
| | 15.3 | 12.6 | 12.8 | 80 | 72 | 92 | +12 | 95 | 65 | 80 |
| | 14.4 | 17.0 | 13.0 | 82 | 61 | 88 | +6 | 60 | 65 | 70 |
| **mean** | **14.6** | **13.5** | **14.6** | **74** | **58** | **82** | **+8** | **94** | **81** | **96** |
| **s.d.** | 0.75 | 4.78 | 2.48 | 11.5 | 11.1 | 12.8 | 3.3 | 18.6 | 26.9 | 25.8 |
| **Days** | **0** | **60** | **90** | **0** | **60** | **90** | **0-90** | **0** | **60** | **90** |
| NO SUPPLEMENT | | | | | | | | | | |
| | 13.8 | 11.5 | 13.1 | 62 | 33 | 31 | -31 | 95 | 75 | 120 |
| | 14.0 | 11.1 | 16.4 | 60 | 51 | 58 | -2 | 100 | 60 | 95 |
| | 15.9 | 16.7 | 14.0 | 45 | 19 | 19 | -26 | 50 | 65 | 75 |
| | 16.0 | 8.0 | 12.1 | 85 | 61 | 65 | -20 | 65 | 50 | 85 |
| | 14.3 | 8.8 | 15.7 | 60 | 42 | 46 | -14 | 65 | 65 | 90 |
| | 13.2 | 14.8 | 13.8 | 94 | 41 | 63 | -31 | 80 | 75 | 100 |
| **mean** | **14.5** | **11.8** | **14.2** | **68** | **41** | **47** | **-21** | **76** | **65** | **94** |
| **s.d.** | 1.16 | 3.38 | 1.61 | 18.2 | 14.5 | 18.7 | 11.3 | 19.3 | 9.5 | 15.3 |
| **SED** | 0.54 | 2.33 | 1.18 | 8.6 | 7.3 | 9.1 | 4.7 | 10.7 | 11.4 | 12.0 |
| **Sig P** | NS | NS | NS | NS | .05 | .01 | .01 | NS | NS | NS |

**Discussion**

Despite the low herbage concentrations of selenium these cattle were of adequate selenium status at transfer to grass and although the non supplemented group showed a fall in GSHPx activities they were all of adequate status at the end of the grazing season. This situation was probably due to giving purchased feedstuffs during the winter to which trace elements were added. This increased the selenium status to such a level that even the consumption of inadequate herbage did not reduce the animals to a state of deficiency.

With the herbage cobalt, the situation was reversed with an adequate intake (0.11 mg/kg in the herbage) giving plasma Vitamin $B_{12}$ concentrations which would be classed as deficient. Despite this deficient status the cattle had a mean liveweight gain over the 90 days of 0.89 kg/day.

In conclusion, administration of the MTEB maintained the selenium status whilst the non supplemented group fell and as with previous experiments no significant increase in plasma Vitamin $B_{12}$ was observed by supplementing animals with the MTEB.

## Experiment 3.9

## The use of MTEB in yearling cattle grazing a known selenium and cobalt deficient site.

### Introduction

This site had a history of clinical and subclinical selenium deficiency. Whole blood glutathione peroxidase activities as low as 2 iu/ml PCV had been recorded. Routine blood sampling also revealed a situation of marginal cobalt deficiency.

### Materials and methods

Twenty-five Limousin and Romangola cross heifer and steer cattle aged 12 months were used in this experiment. Because of the extensive previous history of clinical selenium deficiency only six animals received no supplement with the remaining 19 being given two MTEB at transfer to grass in the spring. The cattle were divided at random into the two groups. The MTEB given here were those with the increased density. Blood samples were taken from all animals at the start of the trial and on another two occasions with the last being 132 days after bolus administration. The blood samples were assayed for plasma copper and plasma Vitamin $B_{12}$ concentrations and for whole blood glutathione peroxidase (GSHPx) activities. Herbage samples were taken in late July and analysed for copper, molybdenum, sulphur, selenium, cobalt and iron.

### Results

The herbage analyses are shown in Table 3.18 and indicate a selenium deficiency with the herbage meeting only 40% of the dietary requirement but copper and cobalt levels were both within the normal range.

**Table 3.18 Trace element analyses of the herbage in Experiment 3.9 mg/kg DM.**

| | |
|---|---|
| Copper | 15.5 |
| Molybdenum | 0.85 |
| Sulphur | 560 |
| Selenium | 0.04 |
| Cobalt | 0.17 |
| Iron | 600 |

Table 3.19 gives the mean plasma copper concentrations, whole blood GSHPx activities and plasma Vitamin $B_{12}$ concentrations for the two groups of cattle throughout the trial. At the start of the trial, eleven (i.e. 44%) of the animals had plasma copper values which were classed as of marginal status. After 80 days at grass however all values from both groups were within the adequate range of 9.4-24 umol/litre. These adequate values were maintained to the end of the trial. The mean GSHPx activity on day 0 for all the animals was 5 iu/ml PCV (s.d. 2.8). With adequate values being greater than 17 units this was indicative of a severe selenium deficiency. The group receiving no supplement showed only small increases in GSHPx activity over the period of the experiment and remained deficient with the final mean value being 9 iu/ml PCV. The group receiving the MTEB had significantly higher GSHPx activities on day 80 (29 iu/ml PCV) and on day 132 (47 iu/ml PCV) with an overall mean increase from day 0 to day 32 of 42 units. All animals given the MTEB had adequate blood values within 80 days of administration.

At the start of the experiment the overall mean plasma Vitamin $B_{12}$ concentration was 150 ng/litre (s.d. 22.7). This value is within the marginally deficient/deficient range. On day 80 all the animals had values within the range 50-75 ng/litre representing a large reduction in Vitamin $B_{12}$ concentrations from those recorded on day 0. By the end of the experiment 132 days after bolus administration, the mean plasma Vitamin $B_{12}$ concentrations had increased. For the group given the MTEB the value was similar to that recorded on day 0 but the non-supplemented group showed a decrease from 142 to 98 ng/litre by day 132. This resulted in the MTEB group having a significantly greater mean plasma Vitamin $B_{12}$ concentration at day 132 (156 v 98 ng/litre).

**Discussion**

Administration of the MTEB significantly increased the selenium status of the growing cattle on this site. From an initial deficient status adequate GSHPx activities were attained within 80 days for those given the MTEB whilst those given no supplement remained deficient throughout the experiment.

After 80 days, supplementation with the MTEB had no effect on plasma Vitamin $B_{12}$ concentrations. In fact, concentrations had fallen by around 100 ng/litre. At the end of the trial there was a significantly higher mean Vitamin $B_{12}$ concentration in the MTEB supplemented group but the majority of values remained within the deficient range. The validity of this response, when none was recorded after 80 days, must be questioned.

**Table 3.19 The mean plasma copper, whole blood GSHPx and plasma Vitamin $B_{12}$ values for growing cattle given the MTEB or no supplement.**

| | | Copper umol/litre | | | GSHPx iu/ml PCV | | | Vitamin $B_{12}$ ng/litre | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **DAYS** | | **0** | **80** | **132** | **0** | **80** | **132** | **0** | **80** | **132** |
| MTEB | mean | 9.7 | 16.6 | 17.8 | 5 | 29 | 47 | 154 | 53 | 156 |
| n=19 | s.d. | 2.13 | 2.54 | 4.01 | 3.0 | 6.4 | 9.7 | 22.3 | 6.9 | 47.7 |
| NO SUPPLEMENT | mean | 9.7 | 14.3 | 17.9 | 6 | 11 | 9 | 142 | 50 | 98 |
| N=6 | s.d. | 1.41 | 1.84 | 2.42 | 2.3 | 3.3 | 3.3 | 23.4 | 0* | 27.3 |
| **SED** | | 0.76 | 0.95 | 1.35 | 1.17 | 2.00 | 2.61 | 10.8 | - | 15.6 |
| **Sig P** | | NS | 0.05 | NS | NS | 0.001 | 0.001 | NS | - | 0.01 |

* All animals in the no supplement group had values of 50 ng/litre.

## Experiment 3.10

## Comparison of the blood responses of calves given either no supplement, the multiple trace element bolus (MTEB), the soluble glass bolus (SGB) or a MTEB containing double the normal quantities of cobalt and selenium (MTEB SC+).

### Introduction

Two standard multiple trace element boluses in combination with deficient herbage should supply sufficient cobalt and selenium to meet the daily allowance of a 500 kg animal for these nutrients. However the results from the previous experiments indicate that in some cases the supply of cobalt from the MTEB was not sufficient to increase plasma Vitamin $B_{12}$ concentrations above those of animals given no supplement. It was considered that an increased supply of cobalt might give improved plasma Vitamin $B_{12}$ responses. Similarly, although the administration of the MTEB had been shown to supply sufficient selenium to raise whole blood glutathione peroxidase activities to within the normal range on all deficient sites it was considered that increasing the supply of selenium might also be advantageous.

This experiment investigated the use of an alternative MTEB formulation which contains double the standard quantities of cobalt sulphate and sodium selenite. Testing in fistulated cows showed no significant difference in release rate from that of the standard MTEB. This finding was in contrast to the results of Experiment 2.5. This new formulation MTEB(SC+) was compared with the standard MTEB and the recently re-introduced soluble glass bolus (COSECURE, Coopers Pitman Moore Ltd.)

### Materials and methods

Twenty-five Charolais cross steers and heifer single suckling calves aged six months and weighing approximately 250 kg were used in this experiment. The calves grazed the same site as in Experiments 3.4 and 3.5 where marginal selenium deficiency had been recorded in previous grazing seasons.

On the 4th of September they were allocated at random to four groups. Three groups of six calves received either two standard MTEB, two SGB or two MTEB(SC+). The fourth group consisted of seven calves and these received no supplement.

The dimensions and the quantities of copper, cobalt and selenium supplied by the three types of bolus are given in Table 3.20. Both MTEB formulations had a base weight incorporated as described in Experiment 3.7 to increase the bolus density and improve retention. Plate 14 shows the soluble glass bolus and the cardboard sleeve.

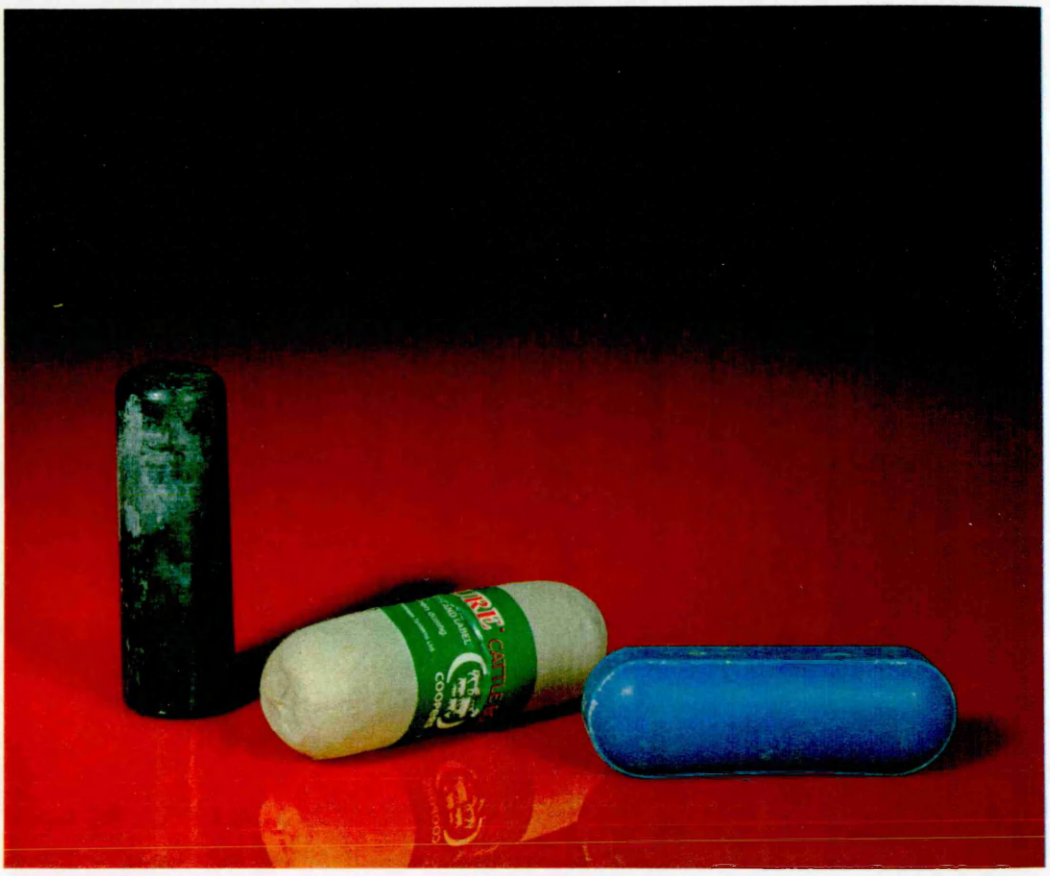
**Table 3.20 The dimensions and estimated amounts of trace elements supplied by two of each of the MTEB, SGB and MTEB(SC+) boluses.**

| | MTEB | SGB | MTEB(SC+) |
|---|---|---|---|
| Daily supply (mg) | | | |
| Copper | 160 | 74 | 160 |
| Cobalt | 0.7 | 2.8 | 1.4 |
| Selenium | 0.8 | 1.7 | 1.6 |
| Length (mm) | 70 | 82 | 70 |
| Diameter(mm) | 26 | 26 | 26 |
| Weight (g) | 105 | 100 | 105 |
| Density ($g/cm^3$) | 2.8 | 2.8 | 2.8 |
| Active life (days) | 240 | 180 | 240 |

Blood samples were taken at bolus administration and at regular intervals until the calves were weaned on 16 November. The blood samples were assayed for plasma copper and plasma Vitamin $B_{12}$ concentrations and for whole blood glutathione peroxidase (GSHPx) activities.

**Plate 14.**

**The re-introduced soluble glass bolus and the cardboard sleeve. On the left is the original type which was withdrawn in 1986.**



## Results

The mean plasma copper concentrations, whole blood GSHPx activities and plasma Vitamin $B_{12}$ concentrations for the four groups are shown in Tables 3.21, 3.22 and 3.23 respectively.

### Plasma copper

The mean initial concentration for all 25 calves was 14.9 umol/litre (s.d. 2.55). This was within the adequate range (9.4 umol - 22 umol/litre). All the calves remained within this normal range for the period of the experiment and no significant effects of supplementation with any of the bolus products were observed.

**Table 3.21 The mean plasma copper concentrations (umol/litre) over 75 days of calves given either no supplement, the MTEB, SGB or MTEB(SC+).**

| DAYS | No supplement | | MTEB | | SGB | | MTEB(SC+) | |
|---|---|---|---|---|---|---|---|---|
| | mean | s.d. | mean | s.d. | mean | s.d. | mean | s.d. |
| **0** | 15.0 | 2.10 | 14.0 | 1.37 | 14.8 | 4.04 | 15.7 | 2.47 |
| **14** | 14.7 | 2.76 | 16.1 | 1.32 | 14.4 | 1.91 | 16.5 | 2.68 |
| **26** | 14.3 | 2.42 | 14.0 | 1.15 | 14.5 | 1.49 | 14.1 | 2.11 |
| **40** | 13.3 | 2.53 | 14.4 | 0.96 | 14.6 | 1.78 | 15.2 | 1.90 |
| **75** | 12.7 | 1.93 | 16.2 | 1.63 | 15.5 | 1.81 | 14.1 | 1.87 |

As no regular trend was observed and as all the mean values are in the normal range no further statistical estimate has been made.

**Whole blood GSHPx**

The mean initial whole blood GSHPx activity for all the calves was 18 u/ml PCV (s.d. 5.3). Ten calves however had values below 17 units and the selenium status of the herd was assessed as being marginally deficient.
The mean change in GSHPx activities for the four groups over the 75 day period were -3, +23, +41 and +42 units/ml PCV for the no supplement, MTEB, SGB and MTEB(SC+) treatments respectively. All bolus products showed significant increases in GSHPx ($P<0.001$) over the no supplement group. The MTEB(SC+) and SGB treatments gave significantly greater increases than the standard MTEB ($P<0.01$).

**Table 3.22 The mean whole blood glutathione peroxidase activities (iu/ml PCV) over 75 days of calves given either no supplement, the MTEB, SGB or MTEB(SC+).**

| DAYS | No supplement | | MTEB | | SGB | | MTEB(SC+) | |
|---|---|---|---|---|---|---|---|---|
| | mean | s.d. | mean | s.d. | mean | s.d. | mean | s.d. |
| **0** | 17 | 5.4 | 21 | 7.1 | 18 | 5.7 | 20 | 2.8 |
| **14** | 16 | 6.6 | 22 | 6.8 | 20 | 7.3 | 24 | 3.4 |
| **26** | 11 | 2.6 | 21 | 6.5 | 27 | 9.2 | 36 | 7.0 |
| **40** | 10 | 1.3 | 21 | 5.0 | 31 | 5.7 | 28 | 3.3 |
| **75** | 14 | 3.9 | 44 | 6.6 | 59 | 7.7 | 62 | 9.6 |
| **Change** | | | | | | | | |
| **0-75** | **-3** | 7.3 | **23** | 8.9 | **41** | 10.6 | **42** | 4.6 |

Significance of 0-75 days change.

| | SED | Sig P |
|---|---|---|
| Nil v MTEB | 4.70 | 0.001 |
| Nil v SGB | 5.26 | 0.001 |
| Nil v MTEB(SC+) | 3.51 | 0.001 |
| MTEB v SGB | 5.65 | 0.01 |
| MTEB v MTEB(SC+) | 4.08 | 0.01 |

## Plasma Vitamin $B_{12}$

The cobalt status of the site had not been assessed previously but with liveweight gains of over 1.0 kg/day from birth to weaning there was no indication of there being a deficiency problem. However, the mean initial plasma Vitamin $B_{12}$ concentration for all calves was 116 ng/litre (s.d. 37.5) and 24 of the calves had values classed as deficient and only one calf had a plasma concentration above the 200 ng/litre threshold for adequate status.

Over the period of the experiment the unsupplemented group showed large increases in plasma Vitamin $B_{12}$ status such that by day 75 only two animals were deficient and these (175 and 185 ng/litre) were classed as marginally deficient.

The mean changes over the 75 day experimental period for the four groups were +151, +213, +183, +226 ng/litre for the no supplement, MTEB, SGB and MTEB(SC+) respectively. There were no significant differences between the groups.

**Table 3.23 The mean plasma Vitamin $B_{12}$ (ng/litre) concentrations over 75 days of calves given either no supplement, the MTEB, SGB or MTEB(SC+).**

| DAYS | No supplement | | MTEB | | SGB | | MTEB(SC+) | |
|---|---|---|---|---|---|---|---|---|
| | mean | s.d. | mean | s.d. | mean | s.d. | mean | s.d. |
| **0** | 101 | 34.2 | 128 | 40.5 | 96 | 16.9 | 140 | 42.0 |
| **14** | 133 | 32.9 | 194 | 77.4 | 149 | 38.7 | 160 | 36.1 |
| **26** | 161 | 40.4 | 268 | 45.0 | 222 | 94.2 | 280 | 68.7 |
| **40** | 198 | 45.5 | 272 | 100.0 | 269 | 54.2 | 323 | 60.1 |
| **75** | 253 | 1.5 | 341 | 86.3 | 279 | 93.4 | 366 | 115.7 |
| **Change** | | | | | | | | |
| **0-75** | **151** | 54.1 | **213** | 76.9 | **183** | 78.4 | **226** | 80.2 |

Significance of 0-75 days change

| | SED | Sig P |
|---|---|---|
| Nil v MTEB | 38.4 | NS |
| Nil v SGB | 38.9 | NS |
| Nil v MTEB(SC+) | 39.5 | NS |
| MTEB v SGB | 44.8 | NS |
| MTEB v SGB(SC+) | 45.3 | NS |

**Conclusion**

The increased supply of selenium from the SGB and the MTEB(SC+) over that of the standard MTEB (1.7 and 1.6 v 0.8 mg/day) gave improved responses in GSHPx activities.

However the increased supply of cobalt from the SGB and MTEB(SC+) over that of the standard MTEB (2.8 mg and 1.4 mg v 0.7 mg/day) did not give improved responses in plasma Vitamin $B_{12}$ concentrations. The reasons for this are unclear since the claimed 2.8 mg/day supplied by the SGB represents around four times the daily allowance (MAFF et al., 1983) for the liveweight of animal used in this experiment.

# SECTION 4

## The inclusion of other substances within the bolus matrix.

The University of Glasgow patented bolus, being simple to manufacture and leaving no permanent residue in the rumen, is a useful vehicle for the intraruminal supply of other biologically active substances. This section describes the inclusion of vitamin, anthelmintic and growth promoting antibiotic substances within the bolus matrix.

# SECTION 4

**Experiment 4.1**
**The inclusion of the anthelmintic ivermectin within the bolus matrix.**

**Experiment 4.2**
**The inclusion of the anthelmintic levamisole hydrochloride within the bolus matrix.**

**Experiment 4.3**
**The inclusion of high levels of Vitamin E within the bolus matrix.**

**Experiment 4.4**
**The inclusion of the anthelmintic oxfendazole within the bolus matrix.**

**Experiment 4.5**
**The inclusion of laidlomycin propionate within the bolus matrix.**

**Experiment 4.1.**

**Inclusion of the anthelminitc ivermectin within the bolus matrix.**

## Introduction

Ivermectin is a member of the Avermectins group of anthelmintic substances and is produced by the actinomycete Streptomyces avermitilis. It has wide antiparasitic and insecticidal properties (Campbell & Benz, 1983) and with continuous intraruminal administration only about 40 ug/kg liveweight/day (Preston, J.M. 1987) are required for parasitic control. Thus the required dose of ivermectin for an animal of say 200 kg is only 8 mg/day.

This has obvious advantages for incorporation into a bolus since the inclusion level will be low and hopefully therefore not adversely affect bolus release rate. An early prototype of the trace element bolus described by Simpson (1985) including ivermectin has been shown to control Ostertagia ostertagi and Cooperia oncophora (Preston et al., 1987).

This work involved calves weighing 200 kg which were housed and fed hay and concentrates. The mean release rate of the boluses over 77 days was 0.14 g/bolus/day and with a 1.4% inclusion of ivermectin this released 1.96 mg/bolus/day which equates to a total of 3.92 mg/animal or a dose of 20 ug/kg. That is approximately half the estimated required dose. This experiment describes the inclusion of ivermectin into the trace element bolus whose formulation is described in Section 1.4.

## Materials and methods

These were as in Section 1.4 with the composition being changed to:

| | g/100 g |
|---|---|
| copper oxide powder | **25.0** |
| standard mix | **68.4** |
| zinc sulphate heptahydrate | **5.0** |
| ivermectin | **1.6** |

Assuming a release rate of 0.25 g/bolus/day the inclusion of 1.6% ivermectin should give 8 mg ivermectin/animal/day.

In Experiment 4.1A, three fistulated cows each received a pair of boluses and the test period was 91 days. From days 0-56 the cows were at grass and thereafter hay and 2 kg concentrates were fed. In Experiment 4.1B two fistulated cows each received a pair of boluses and the test period was 42 days. The cows were fed hay and 2 kg concentrate for the whole of that period.

The boluses for experiments 4.1A and 4.1B were made in two separate batches about 6 months apart. The boluses all weighed 100 g.

## Results

The release rates for boluses in Experiments 4.1A and 4.1B are given in Table 4.1.

In Experiment 4.1A the mean release rate over the 91 day test period was 0.18 g/bolus/day. There was no significant change in release rate (0.19 g v 0.17 g) when the cows were transferred from grass to hay and concentrates. This is a similar finding to the results with the unsupplemented trace element bolus shown in Table 1.2. The release rate was about 70% of that required to give the target 8 mg/head/day of ivermectin.

In Experiment 4.1B the release rate of the boluses over days 0-42 was significantly greater than those in Experiment 4.1A over the same period (0.34 g v 0.18 g, $P<0.01$). Also the variation in release rate in the same period between boluses was less in Experiment 4.1B than in Experiment 4.1A. The coefficient of variation for the release rates over days 0-42 were 10% (Experiment 4.1B) and 33% (Experiment 4.1A). Over the 42 day test period the boluses in Experiment 4.1B more than met the target release rate needed to achieve 8 mg/animal/day of ivermectin.

**Table 4.1 The mean release rate (g/bolus/day) of boluses containing ivermectin in Experiments 4.1A and 4.1B.**

| | | Expt. 4.1A* n=6 | | Expt. 4.1B** n=4 | |
|---|---|---|---|---|---|
| | | mean | s.d. | mean | s.d. |
| **Day** | | | | | |
| | **0-7** | 0.26 | 0.081 | 0.67 | 0.140 |
| | **8-14** | 0.19 | 0.044 | 0.25 | 0.092 |
| | **15-21** | 0.16 | 0.069 | 0.31 | 0.102 |
| | **22-28** | 0.14 | 0.051 | 0.27 | 0.060 |
| | **29-35** | 0.16 | 0.054 | 0.26 | 0.054 |
| | **36-42** | 0.20 | 0.088 | 0.29 | 0.080 |
| | **0-42** | **0.18** | 0.059 | **0.34** | 0.034 |
| **mean weight at day** | **42** | **93.9** | 2.84 | **87.5** | 1.35 |
| | **43-49** | 0.19 | 0.076 | | |
| | **50-56** | 0.20 | 0.066 | | |
| | **57-63** | 0.23 | 0.049 | | |
| | **64-70** | 0.11 | 0.029 | | |
| | **71-77** | 0.18 | 0.069 | | |
| | **78-84** | 0.17 | 0.056 | | |
| | **85-91** | 0.17 | 0.159 | | |
| **Release rate over days** | | | | | |
| | **0-56** | **0.19** | 0.060 | | |
| | **56-91** | **0.17** | 0.038 | | |
| | **0-91** | **0.18** | 0.051 | | |
| **mean weight at day** | **56** | **91.1** | 3.73 | | |
| | **91** | **85.3** | 5.03 | | |

* Experiment 4.1A days 0-56 at grass days 57-91 on hay and concentrates.
** Experiment 4.1B days 0-42 on hay and concentrates.

Significance of difference Experiments 4.1A and 4.1B

SED 5.71 $P<0.01$

The reasons for the difference in results between Experiments 4.1A and 4.1B are unclear. So far as a possible difference due to change of diet is concerned in Experiment 4.1A no significant effect on release rate was shown when cows were changed from grass to hay and concentrates. Similarly the results in Table 1.2 for Matrix No 1 confirm this and also show no effect from a change of diet in the other direction, i.e. from hay and concentrates to grass.

It may be that with ivermectin inclusion the boluses released less when the cattle were at grass and this period of slow release conditions the bolus such that even when changed to a diet of hay and concentrate the release rate stays low. If this is so the results of Experiment 4.1B may be the appropriate results for animals fed hay and concentrates from the start.

However another possible reason for the difference may be that the boluses were made and tested some time apart and the processing and mixing of materials and even the materials themselves may have been slightly different.

It can be concluded that ivermectin can be incorporated into the trace element bolus matrix without difficulty and the boluses release amounts of ivermectin in the order required over extended periods of time. Variation in release rates between boluses and between batches were however high and this would need to be further investigated at some stage if a large scale production process were to be envisaged.

## Experiment 4.2.

## Inclusion of the anthelmintic levamisole hydrochloride within the bolus matrix.

### Introduction

Levamisole hydrochloride is a broad spectrum anthelmintic widely used in cattle and sheep for the control of nematode infections. For cattle it can be given by three routes; oral, subcutaneous and as an application to the skin of the back. (Forsyth, 1968; Baker & Fishe, 1972; Rowlands & Berger, 1977). The recommended therapeutic treatment dose for the first two methods is 7.5 mg/kg liveweight and for the third 10 mg/kg liveweight.

As yet there is not a rumen bolus formulation for levamisole hydrochloride which has received a veterinary product licence in the United Kingdom. The daily dose likely to be required for cattle when given from a slow release bolus was estimated at 2 mg/kg (Armour, 1987). At this level the required dose per day is much greater than for ivermectin (40 ug/kg) although the ratio of continuous to single therapeutic dose is similar. Therefore the inclusion level of levamisole hydrochloride within the bolus must be correspondingly higher if the bolus is to be of similar weight and expected life.

Since these present experiments were conducted a dose titration study by Parkins et al. (1988) has established the required daily dosage for continuous administration of levamisole hydrochloride for sheep infected with Ostertagia circumcincta to be in excess of 3 mg/kg/day. This is roughly half the recommended single oral therapeutic dose and as such the economic benefits of a continuous release levamisole hydrochloride bolus must be questioned.

However this evidence was not available at the time of these present experiments. Two experiments were conducted. The first examined the release rate in fistulated cows of 25 and 19 mm diameter boluses and the second involved non-fistulated sheep given a 19 mm diameter bolus.

## Experiment 4.2A

### Materials and methods

These were as in Section 1.5 with the bolus composition being:

| | g/100 g |
|---|---|
| copper oxide needles | **25** |
| standard mix | **50** |
| levamisole hydrochloride | **25** |

Copper oxide needles were used as the change to copper oxide powder had not yet taken place.

Two types of boluses with different diameters were made, one at the standard 25 mm cattle size and another at 19 mm, the sheep size. The 25 mm boluses weighed 100 g and the 19 mm diameter boluses weighed 35 g. Both were tested in pairs in fistulated cows. The test period was 49 days and the cows were given hay and 2 kg of concentrates over the whole of that period.

### Results

Table 4.2 shows the release rates of the 25 mm and 19 mm diameter boluses. The 25 mm bolus showed a constant release of material with a mean release rate of 0.47 g over days 0-49. The variation in release rate between boluses over that period was small, i.e. coefficient of variation was only 7.9%. At that release rate, it can be calculated that 235 mg of levamisole hydrochloride will be released, i.e. 60% of the estimated dose for an animal of 200 kg. The estimated life of the 25 mm diameter bolus would be 210 days.

The mean release rate of the 19 mm bolus over days 0-49 was 0.17 g and it also showed a fairly constant release pattern, apart from days 43-49 where the release rate fell to 0.10 g. The variation in release rate over the whole period was higher than for the 25 mm diameter bolus (c.v 23%). The calculated release of levamisole hydrochloride was 85 mg. This dose would be suitable for sheep of up to 40 kg liveweight, and a 35 g bolus of this formulation would have an expected life of 206 days.

**Table 4.2 The mean release rate (g/bolus/day) of 25 mm (100 g) and 19 mm (35 g) diameter boluses containing levamisole hydrochloride.**

| | Diameter | | | |
|---|---|---|---|---|
| | 25 mm | | 19 mm | |
| Day | mean | s.d. | mean | s.d. |
| **0-7** | 0.45 | 0.119 | 0.27 | 0.068 |
| **8-14** | 0.52 | 0.159 | 0.20 | 0.101 |
| **15-21** | 0.35 | 0.070 | 0.17 | 0.038 |
| **22-28** | 0.34 | 0.027 | 0.16 | 0.040 |
| **29-35** | 0.39 | 0.085 | 0.14 | 0.041 |
| **36-42** | 0.42 | 0.051 | 0.15 | 0.028 |
| **43-49** | 0.37 | 0.066 | 0.10 | 0.013 |
| **0-49** | **0.47** | 0.037 | **0.17** | 0.039 |
| **bolus weight at day 49** | **77.1** | 1.91 | **26.6** | 1.90 |
| **levamisole HCl mg/day** | **235** | | **85** | |

The exposed surface area at the active end of the 25 mm diameter bolus is 4.91 $cm^2$ and that for the 19 mm diameter bolus is 2.26 $cm^2$, a ratio of 2.17:1. The overall mean daily losses from the two types of bolus (Table 4.2) were 0.47 and 0.17 g respectively, a ratio of 2.76:1. Accordingly the rate of release of the smaller bolus is somewhat slower than expected on grounds of surface area alone which perhaps indicates the relative importance of mutual erosion rather than dissolution of material.

In practice, the life of both boluses would need to be reduced to nearer 100-120 days. This could be readily achieved by reducing the bolus weight and length.

## Experiment 4.2B

The results obtained with the 19 mm diameter sheep-sized bolus in Experiment 4.2A were sufficiently encouraging for a further experiment to be conducted.

### Materials and methods

The composition of the boluses were as for Experiment 4.2A, i.e. 25% copper oxide needles, 50% standard mix and 25% levamisole hydrochloride. The density of the boluses was 2.2 g/cm$^3$.

Thirteen adult Suffolk-cross wether sheep were used. All were given a single bolus and were then transferred to grass. The intention was to slaughter 4 sheep after 20 days, a further 4 sheep at day 51 and the remaining 5 sheep at day 65. In the event when the boluses were recovered from the 4 sheep at day 20 the mean release rate was found to be very low. In consequence on day 20 the remaining 9 sheep were given an additional bolus.

At slaughter the reticulo-rumen was thoroughly searched and the boluses recovered. A sample of liver was obtained from each sheep for copper analysis.

### Results

For the four sheep slaughtered at day 20 all the boluses were recovered. The mean daily loss in weight of 0.04 g was irregular from sheep-to-sheep and corresponded to a release of only 10 mg levamisole hydrochloride in relation to the target release of 40-80 mg. In an attempt to increase the rate of dissolution a further bolus was administered to all the sheep on day 20. These boluses were marked so that they could be identified from those given originally.

At day 50 two boluses were recovered from each of three sheep and one bolus from the fourth sheep. This latter bolus was from the original batch given on day 0 and had lost only 0.03 g/day over 50 days in contrast to the others with a mean loss of 0.20 g per bolus when they were recovered as pairs.

At day 65 pairs of boluses with an overall mean daily loss in weight of 0.19 g were recovered from three sheep. Only one bolus was present in each of the other two sheep, one of which had been administered at day 0 and the other at day 20.

Of the 22 boluses which were administered to 13 sheep three boluses were not recovered. It is possible (but very unlikely) that these were not discovered at slaughter. The loss is perhaps a reflection of the low density of these boluses (2.2 g $cm^3$).

**Table 4.3 The mean release rate (g/bolus/day) of 35 g boluses containing levamisole hydrochloride given to 13 sheep and their liver copper concentrations at slaughter.**

| | | Release rate | | Liver copper mg/kg DM |
|---|---|---|---|---|
| Slaughter at | No.of sheep | Days 0-20 | | |
| Single bolus at day 0 | | | | |
| **Day 20.** | 4 | 0.010 | | 36 |
| | | 0.078 | | 90 |
| | | 0.024 | | 61 |
| | | 0.055 | | 23 |
| Additional bolus at day 20 | | | | |
| | | Days 0-50 | Days 20-50 | |
| **Day 50.** | 4 | 0.165 | 0.254 | 733 |
| | | 0.161 | 0.199 | 745 |
| | | 0.216 | 0.211 | 260 |
| | | 0.033 | N.R. | 86 |
| **Day 65.** | 5 | 0.184 | 0.198 | 990 |
| | | 0.191 | 0.241 | 786 |
| | | 0.133 | 0.180 | 540 |
| | | 0.028 | N.R. | 433 |
| | | N.R. | 0.193 | 305 |

N.R.not recovered at slaughter.

The mean loss of approximately 0.20 g per bolus represents a combined daily release from two boluses of 98 mg levamisole hydrochloride. It is estimated that the life of the 35 g boluses would be about 180 days. If indeed 40-80 mg levamisole hydrochloride/day is appropriate for lambs growing from about 20 to about 40 kg liveweight the release rate could perhaps be reduced by the inclusion of a lower concentration of levamisole hydrochloride in the bolus matrix.

However, the relatively high rate of non-recovery of the boluses and the anticipated cost relative to the more normal methods of administration by injection or oral dosing were such that this work was not continued. The technique would however be useful as an experimental model.

The copper content of the boluses was such that from a mean release rate in weight when pairs of boluses were present of 0.20 g/bolus/day, 78 mg Cu was provided per sheep per day. Table 4.3 indicates that at day 20 when a mean of only about 8 mg Cu per day would have been provided by the single bolus the mean liver copper content was about 50 mg/kg DM. By day 50 and day 65 when pairs of boluses were present this had increased to about 675 mg Cu/kg DM. Individual values were rather less where only one bolus was recovered and where it was uncertain for how long two boluses had been present and active.

This is perhaps an unacceptably high rate of copper accumulation and this is an aspect to be considered where copper oxide is included as a constituent of a sheep bolus.

## Experiment 4.3

### Inclusion of high levels of Vitamin E within the bolus matrix.

### Introduction

The term Vitamin E refers to a group of lipid soluble compounds whose main function is an antioxidant. Alpha tocopherol is the most active of these compounds. In its' role as an antioxidant Vitamin E acts in synergism with the trace element selenium (Diplock, 1981).

Where Vitamin E and selenium are deficient or where there are high dietary intakes of polyunsaturated fatty acids then skeletal and cardiac myopathies may occur, for example white muscle disease. This condition generally occurs in young ruminants.

In adult ruminants responsive disorders also occur for example in the incidence of retained placentae in dairy cows and has been shown to be responsive to Vitamin E and selenium supplementation. (Trinder et al., 1973; Julien et al., 1976; Julien, Conrad & Moxon, 1976; Harrison, Hancock & Conrad, 1984). It was during investigations into retention of the placenta that a reduction in clinical mastitis was observed in the supplemented animals (Smith et al., 1984). The treatments were either an oral supply of 1000 mg Vitamin E daily for the dry period together with an intramuscular injection of selenium given 21 days prepartum (at 0.1 mg/kg liveweight as sodium selenite), or Vitamin E alone, or selenium alone or no treatment.

Vitamin E supplementation reduced the incidence of clinical mastitis by 37%. No effect of selenium alone or any interaction with Vitamin E on mastitis incidence was reported. However the duration of clinical symptoms was reduced by 46% for Vitamin E given alone and 62% for the combined treatment. This was the first observation that Vitamin E and selenium supplementation could influence the incidence and duration of mastitis in dairy cows. A second trial (Smith et al., 1985) also showed improvements in mastitis control by supplementation with Vitamin E and selenium with 42% reduction in infection at calving, 59% reduction in duration of infection and a 32% reduction in clinical mastitis over the whole lactation. The milk somatic cell count was also lower at day 14 of the lactation and for the whole lactation.

In recent work (Weiss et al., 1990) where nine commercial herds were monitored the influence of Vitamin E and selenium on mastitis was further confirmed. High serum selenium values were associated with reduced clinical mastitis and low bulk milk somatic cell counts and the concentration of Vitamin E in the diet were negatively correlated to incidence of clinical mastitis.

The rationale for prepartum supplementation as used in the first two studies has been suggested by Smith (1986) to be that a low plasma Vitamin E concentration occurs at parturition due to the inadequacy of dry cow diets and an independent natural decline over about 30 days prepartum (Smith et al., 1984). This coincides with a particular period of increased susceptibility to mastitis which occurs from two weeks prepartum to early lactation (Smith, Todhunter & Schoenberger, 1985).

The actual mechanism involved in the reduction of clinical mastitis is not known but the improved immuno-responsiveness reported (Tengerdy et al., 1983; Turner, Wheatley & Beck, 1985) from Vitamin E and selenium supplementation may be involved.

Thus it had been shown that supplementing dairy cows 45-60 days prepartum with 1000 mg Vitamin E and selenium improves the Vitamin E and selenium status during a likely period of inadequacy and may reduce the incidence and duration of clinical mastitis which might otherwise occur. During the dry period cows may either be at grass or be housed in groups. In both cases little or no concentrate feed may be given. One method of supplying the quantities of Vitamin E and selenium during the dry period which have been demonstrated to be of benefit to the dairy cow might be to administer them in a rumen bolus which would provide a daily release of sufficient material over that period.

This section describes a number of experiments designed to formulate such a bolus.

**Materials and methods**

The Vitamin E powder used contained 50% Vitamin E. It was a very soluble material and in an attempt to prevent a bolus with a high inclusion rate from dissolving too quickly dicalcium phosphate (DCP) which is virtually insoluble was included in the bolus matrix. The low bulk density of Vitamin E powder also required that there be an increased inclusion of copper oxide powder (40%) to attain a reasonable bolus density.

Six formulations as shown in Table 4.4. with various combinations of DCP were prepared. Sodium selenite was included in all of these at 0.2% with the aim of providing around 2 mg selenium per day from two boluses.

**Table 4.4 The composition (g/100 g) of six bolus formulations containing Vitamin E powder.**

| | **E1** | **E2** | **E3** | **E4** | **E5** | **E6** |
|---|---|---|---|---|---|---|
| Vitamin E | **40** | **30** | **28** | **26** | **24** | **20** |
| Dicalcium phosphate | **19.8** | **29.8** | **31.8** | **33.8** | **35.8** | **39.8** |
| Copper oxide powder | 40 | 40 | 40 | 40 | 40 | 40 |
| Sodium selenite | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |

The boluses were prepared and tested as in Section 1.5 except that the pressure used to form the bolus was $4t/in^2$. All formulations were tested in pairs in each of three non pregnant fistulated cows. The test period was 21 days and the diet for the whole of that period was hay and 2 kg of concentrate.

**Results**

The results for the six bolus formulations tested are shown in Table 4.5. The maximum amount of material which could be placed in the die was 50 g this gave a coated bolus weight of 51 g. The low bulk density of the Vitamin E powder gave a low bolus density ranging from 2.1 g/cm$^3$ to 2.4 g/cm$^3$ as the inclusion of Vitamin E decreased and the inclusion of DCP increased.

Formulations E1 and E2 had dissolved almost completely by day 7 leaving only the resin shell. A small alteration in composition modified the release rate with E3 having a release rate of 2.1 g/day over days 0-7 and 4.6 g/day over days 8-14. This formulation provided 588 mg and 1288 mg respectively from two boluses for the first and second weeks on test. This was close to the target of 500-1000 mg/animal/day but by day 14 only a mean of 5 g remained of the boluses. The selenium provided from the two boluses was 3.8 and 8.3 mg/day this was two to four times the required dose but could be easily adjusted by reducing the inclusion rate of sodium selenite.

The difference in release rate between E3 and E4 is quite remarkable since there is only a difference of 2% Vitamin E/DCP. However experience with the trace element bolus (Experiments 2.2, 2.4 and 2.5) suggests that this is not a unique phenomena. E3 had a release rate of 2.1 g/day over days 0-7 contrasting with the 0.4 g/day for E4. At that latter release rate only 1.4 mg Vitamin E was provided per day from two boluses. This was obviously too low but the testing of the other formulations in the series was continued to examine the effect of inclusion rate of Vitamin E/DCP on bolus release rate. Both E5 and E6 had a release rate of 0.2 g/bolus/day over days 0-7 confirming that altering the Vitamin E/DCP inclusion rate can provide a range of bolus release rates from 6.9 g/bolus/day (E1) to 0.2 g/bolus/day (E6).

**Table 4.5 The dimensions and mean release rate (g/bolus/day) of six bolus prototypes containing Vitamin E powder.**

| Formulation | E1 | E2 | E3 | E4 | E5 | E6 |
|---|---|---|---|---|---|---|
| Initial weight (g) | 51.4 | 51.3 | 51.3 | 51.4 | 51.6 | 51.6 |
| **Density (g/cm3)** | 2.1 | 2.2 | 2.2 | 2.3 | 2.3 | 2.4 |
| **0-7** | 6.9 | 6.7 | 2.1 | 0.4 | 0.2 | 0.2 |
| **8-14** | - | - | 4.6 | 0.3 | <0.1 | 0.2 |
| **15-21** | - | - | - | 0.3 | <0.1 | 0.2 |
| **Expected life (days)** | **7** | **7** | **16** | **150** | **555** | **286** |
| **Release Vitamin E from two boluses mg/day** | | | | | | |
| **0-7** | 2760 | 2010 | 588 | 104 | 48 | 40 |
| **8-14** | | | 1288 | 78 | 12 | 40 |
| **15-21** | | | | 78 | 12 | 40 |
| **Total mg Vitamin E** | **2760** | **2010** | **1876** | **260** | **72** | **120** |

## Conclusions

High levels of Vitamin E (30-40%) in a bolus matrix of DCP and copper oxide powder gives a bolus which dissolves in less than 7 days. This massive release of material can be reduced by increasing the inclusion of DCP at the expense of Vitamin E. A bolus matrix with 28% Vitamin E and 32% DCP (E3) showed most promise. The low bulk density of Vitamin E powder limited the weight of bolus to 50 g giving E3 an expected life of only 16 days. It would be necessary to produce a larger die or increase its diameter to allow a greater fill of material to give a bolus with an expected life of the required 45-60 days.

## Experiment 4.4

## The inclusion of the anthelmintic oxfendazole within the bolus matrix.

### Introduction

Oxfendazole is a member of the benzimidazole group of anthelmintics. It has a broad activity against nematode parasites (Downey, 1976; Armour, Duncan & Reid, 1978). Currently three routes of administration are available for oxfendazole; oral, intraruminal injection and a pulse-release rumen bolus. Oral and intraruminal administration have been shown to have equal activity (Bairden, Armour & Reid, 1983) and the recommended therapeutic dose for both is 4.5 mg/kg liveweight. The oxfendazole pulse release bolus (OPRB) has five tablets each containing 750 mg oxfendazole which are released at intervals of 21 days. (Rowlands, Shepherd & Collins, 1988) The bolus has an initial density of 3 $g/cm^3$ to ensure retention. It is designed for administration to calves weighing between 100 and 200 kg when transferred to grass. Since a fixed quantity of oxfendazole is given on each of the five occasions at 21 day intervals the dose in mg/kg liveweight will be reduced as the animal gains weight over the 105 day active life of the bolus. Consequently the cattle liveweight range for usage is restricted to ensure efficacy.

A recent OPRB modification is a bolus containing six pellets each of 1250 mg oxfendazole, the first of which is released within 24 hours of administration. This allows larger cattle of between 200 and 400 kg to be treated and the 'front loading' allows it to be used in cattle already under parasitic challenge. Treatment with the OPRB has been shown to give effective control of parasitic nematodiases in young grazing cattle (Jacobs et al., 1987; Mitchell, 1987; Downey, 1988).

Oxfendazole was the anthelmintic used in initial studies which showed that high levels of efficacy could be attained by intra-ruminal continuous release. These studies utilised the 'Laby device' (Laby, 1978). This is a controlled release capsule administered orally, which when it enters the reticulo-rumen changes shape to prevent regurgitation.

When this device was administered to cattle (Anderson & Laby, 1979) and the release of oxfendazole from two prototypes was 0.29 and 0.48 mg/kg/day the percentage efficiency against Ostertagia ostertagi adults developing 4th stage and early 4th stage was respectively 68, 57 and 84% for 0.29 mg/kg/day and 99, 87 and 93% for 0.48 mg/kg/day. This compared with a single oral dose of 2.5 mg/kg liveweight which resulted in 92, 74 and 90% control. Thus a continuous daily

dose of 0.48 mg/kg/day of oxfendazole was highly efficient in cattle for the control of all parasitic stages of O.ostertagi.

In a later study (Anderson et al., 1980) with sheep three release rates were examined 0.17, 0.28 and 0.48 mg/kg/day again delivered by the Laby intraruminal capsule. The earlier work with cattle was confirmed with 0.48 mg/kg/day having a high anthelmintic activity and giving increases in liveweight gain and wool production compared with untreated control animals.

Thus oxfendazole can give a high degree of anthelmintic control by continuous daily release when the amount provided is about ten percent of the single effective oral dose. This effect is explained by the prolonged high plasma concentrations and the sustained low level in the gut (Anderson & Laby, 1979) since with benzimidazoles the length of exposure to the drug as well as the concentration is important in determining anthelmintic activity (Pritchard, Hennessy & Steel, 1978).

This section describes a number of experiments designed to formulate a bolus treatment to deliver from two boluses a total of 100 mg oxfendazole per day over 120 days.

## Materials and methods

Four formulations were prepared which incorporated oxfendazole with the principal components of the trace element bolus, i.e. copper oxide powder, manganese sulphate, zinc oxide and zinc sulphate. Initial investigations suggested that concentrations of oxfendazole higher than about 10% led to problems in compression and coating with this type of matrix.

The four formulations are given in Table 4.6. The boluses weighed 60 g and the pressure used to form the bolus was 4 t/in$^2$. Other materials and methods were as in Section 1.5. Six boluses of each formulation were tested in pairs in each of three fistulated cows. The test period was 28 days and the cows diet for the whole of that period was grass silage.

**Table 4.6 The composition (g/100 g) of bolus formulations containing oxfendazole.**

| | OXF 1 | OXF 2 | OXF 3 | OXF 4 |
|---|---|---|---|---|
| | | g/100 g | | |
| Oxfendazole | 5 | 10 | 10 | 10 |
| Copper oxide powder | 25 | 25 | 25 | 25 |
| Zinc oxide | 15 | 0 | 10 | 15 |
| Zinc sulphate | 20 | 15 | 5 | 0 |
| Manganese sulphate | 35 | 50 | 50 | 50 |

## Results

The dimensions and mean release rate of the four formulations are given in Table 4.7. As a result of oxfendazole inclusion the bolus density for all types was below 2.3 g/cm$^3$. This low bolus density probably accounts for the loss of two boluses, one from each of OXF 2 and OXF 4. The bolus lost from the OXF 2 batch was not recovered at day 14 or subsequently. The bolus from the OXF 4 batch was not recovered after day 21. The loss of the other bolus affected the release rate of the remaining OXF 2 bolus, it showed negligible loss from day 14 to day 28. This was also the case with the OXF 4 bolus which lost the other bolus.

With all four bolus formulations there was a large initial loss of materials this being 0.31 g/bolus/day for OXF 1 but 2-3 g/bolus/day for OXF 2-4. Thereafter the release rate was markedly reduced, in OXF 3 this was almost by a factor of ten and by days 22-28 the release rate was below 0.070 g/bolus/day for all four types.

**Table 4.7 The dimensions and mean release rate (g/bolus/day) of four bolus prototypes containing oxfendazole**

| | OXF 1 | | OXF 2 | | OXF 3 | | OXF4 | |
|---|---|---|---|---|---|---|---|---|
| Length (cm) | 5.1 | | 5.1 | | 5.0 | | 4.9 | |
| Density ($g/cm^3$) | 2.1 | | 2.1 | | 2.2 | | 2.3 | |
| **Days** | mean | s.d. | mean | s.d. | mean | s.d. | mean | s.d |
| **0-7** | 0.31 | 0.120 | 3.73 | 1.409 | 2.33 | 1.462 | 2.75 | 1.129 |
| **8-14** | 0.13 | 0.059 | 0.58** | 0.240 | 0.27 | 0.206 | 0.32 | 0.439 |
| **15-21** | 0.11 | 0.065 | 0.21** | 0.128 | 0.04 | 0.020 | 0.18 | 0.175 |
| **22-28** | 0.03* | 0.021 | 0.05** | 0.014 | 0.01* | 0.020 | 0.07** | 0.027 |
| **0-28** | **0.13** | 0.057 | **1.02**** | 0.360 | **0.68** | 0.383 | **0.78** | 0.240 |
| **mean final bolus wt (g)** | **57.9** | 15.77 | **32.5** | 10.14 | **42.4** | 10.74 | **39.8** | 6.66 |

* some boluses showed negligible weight loss

** one bolus was not recovered

With an inclusion rate of 5% the required bolus release rate to give 100 mg oxfendazole is 1.0 g/bolus/day. Formulation OXF 1 had a mean release rate over days 0-28 of only 0.13 g/bolus/day and was thus obviously unacceptable, even excluding that it's release rate was declining throughout the test period. For those formulations with an inclusion of 10% oxfendazole (OXF 2-4) the required release rate is 0.5 g/bolus/day. All provide excess quantities of oxfendazole during the first seven days. OXF 2 and OXF 4 meet the target over days 8-14 but thereafter they join OXF 3 in supplying inadequate amounts and have virtually stopped releasing material by day 28.

**Discussion**

Oxfendazole can be included in a bolus matrix to form a bolus which compresses and coats satisfactorily providing that the inclusion rate does not exceed 10% oxfendazole. The density of such boluses is low and in this test where grass silage was fed 2 out of the 24 boluses were lost, presumably by regurgitation.

The release rate of the boluses was greatest over the first seven days and declined to virtually nil by day 28. Such a pattern had not been recorded in the trace element boluses which have a substantially linear release pattern after day 14.

Increasing the inclusion rate of manganese sulphate appeared to increase the release rate of OXF 2-4 even when 10% oxfendazole was included. OXF 2 had the greatest release rate both initially and over the 28 day test period and this may be due to the high level of zinc sulphate inclusion which is known to be highly soluble.

**Experiment 4.5.**

**The inclusion of laidlomycin propionate within the bolus matrix.**

## Introduction

Laidlomycin is a polyether ionophoric antibiotic (Kitame et al., 1974) whose in vitro and in vivo activity in the alteration of ruminal fermentation is improved by acylation of the primary hydroxyl group (Clark et al., 1982).

Initial studies (Spires & Algeo, 1983) with one such acylation product laidlomycin butyrate (LB) showed that it was a more effective enhancer of propionic acid production than the parent compound and that it inhibited lactic acid production more effectively than monensin or laidlomycin. In the same study, monitoring of the performance of beef feedlot cattle supplemented with LB at 33 mg/kg DM in the feed showed that the mean daily liveweight gain was increased by 8% and feed efficiency was improved by 20%.

Laidlomycin is effective at lower additive rates to feed than other feed additives. For example, recommended concentrations for monensin are 10-40 mg/kg and for 15-30 mg/kg for avoparcin. This was established by using laidlomycin propionate (LP) in six feedlot trials involving over one thousand cattle (Spires et al., 1990). In these trials improvement in performance was recorded within the range 6-12 mg LP/kg DM. Average daily liveweight gain was increased from 1.2 kg/day for control cattle to 1.3 kg/day in those given LP, and this was maximised at an additive rate of 6 mg/kg DM. In addition feed conversion was improved from 9.02 for control cattle to 8.31 and 8.00 kg feed/kg liveweight gain for inclusions of 6 and 12 mg LP/kg feed DM respectively. Feed intake was not affected by LP inclusion. The improvements in performance were generally greater when the cattle were given low energy diets.

Laidlomycin propionate is therefore a highly effective feed additive which can be used at low concentrations and which improves liveweight gain and feed conversion ratio due to its' ability to alter rumen fermentation towards more useful products.

This section describes a series of experiments to formulate a rumen bolus containing laidlomycin propionate. The objective was to produce a two bolus treatment capable of releasing 80 mg/day of laidlomycin propionate over a period of 100 days.

## Materials and methods

Three formulations were prepared with the base matrix being copper oxide, manganese sulphate and zinc oxide. Laidlomycin propionate (LP) was included in the bolus matrix at 7.5, 10 and 15%. Table 4.8 gives the composition of the three formulations.

**Table 4.8 The composition (g/100 g) of three bolus formulations containing laidlomycin propionate.**

| | LP 1 | LP 2 | LP 3 |
|---|---|---|---|
| Laidlomycin propionate | 7.5 | 10 | 15 |
| Copper oxide | 25 | 25 | 25 |
| Manganese sulphate | 60 | 60 | 55 |
| Zinc oxide | 7.5 | 5 | 5 |

The bolus weight was limited to 50 g by the low bulk density of laidlomycin propionate. The pressure used to form the bolus was 6 $t/in^2$ for L1 and L2 but because of problems in ejecting the bolus it was reduced to 4 $t/in^2$ for L3.

Six boluses of each formulation were tested in pairs in each of three fistulated cows over a period of 35 days. The cows were fed grass silage ad lib throughout the test period.

**Table 4.9 The dimensions and mean release rate (g/bolus/day) of three bolus prototypes containing laidlomycin propionate.**

| | LP 1 | | LP 2 | | LP 3 | |
|---|---|---|---|---|---|---|
| length (cm) | 4.0 | | 4.2 | | 4.6 | |
| density (g/cm$^3$) | 2.3 | | 2.2 | | 2.0 | |
| | mean | s.d. | mean | s.d. | mean | s.d. |
| **Days** | | | | | | |
| **0-7** | 1.34 | 0.265 | 2.35 | 0.697 | 2.48 | 0.448 |
| **8-14** | 0.84 | 0.808 | 0.36 | 0.138 | 0.33 | 0.097 |
| **15-21** | 0.36 | 0.157 | 0.20 | 0.064 | 0.11 | 0.071 |
| **22-28** | 0.27 | 0.210 | 0.11 | 0.057 | 0.05 | 0.011 |
| **29-35** | 0.19 | 0.085 | 0.10 | 0.065 | 0.05 | 0.037 |
| **0-35** | **0.57** | 0.145 | **0.66** | 0.189 | **0.55** | 0.057 |
| **mean final bolus wt (g)** | **31.3** | 5.09 | **27.6** | 6.54 | **31.9** | 1.99 |

## Results

The bolus density for L1 - L3 was in the range 2.0-2.3 g/cm$^3$ and in contrast to the oxfendazole containing boluses in Experiment 4.5 this did not lead to the loss of the boluses by regurgitation.

The mean release rates for the formulations are given in Table 4.9. To achieve the target of 80 mg/head/day of LP the boluses of L1, L2 and L3 would require to have a release rate of approximately 0.54 g, 0.40 g and 0.27 g/bolus/day respectively. Over the first seven days all three bolus formulations had substantially greater release rates than were recorded over the rest of the test period. With L1 the difference was only a factor of 1.6 but with L2 and L3 it was closer to seven fold. This trend of reducing weight loss continued throughout the trial period for all formulations. By days 29-35 the release rates were only 2-15% of the intial period. Because of this, none of the formulations met the target release of LP for more than the first 14 days.

## Discussion

Laidlomycin propionate can be included with the bolus matrix with a range of inclusion rates. Some difficulties in ejecting the bolus after compression are however encountered with an inclusion rate of 15% but this was overcome by reducing the pressure applied. All formulations tested showed a reduction in release rate over the test period.

The low density and short length of the boluses may have led to reduced interaction, resulting in this effect. However there was a trend for the higher inclusion rates of LP to show it to a greater degree. This effect would need to be investigated to allow the project to proceed.

## SECTION 5

## The development of a trace element bolus for sheep

### Introduction

In many circumstances sheep receive less supplementary feeding than cattle and are therefore more dependant on herbage to supply adequate amounts of trace elements. The potential benefit of a trace element bolus suitable for sheep is considerable. However although in this present thesis a sheep bolus had been developed along with a cattle bolus, the latter has been given greater priority.

This is as a result of the many problems involved with a trace element bolus for sheep. The two main problems in formulating such a bolus are firstly that it be of sufficient density to be retained, regurgitation being a far more serious problem in sheep than cattle (Poole & Connolly, 1967; Millar et al., 1988), and secondly that it provide sufficient copper to supplement deficient diets in the face of antagonist compounds but still contain low enough levels to avoid copper toxicity, again a problem encountered more in sheep than in cattle.

There is also the problem during development of such a bolus of finding a suitable test animal. The rumen fistulation of sheep only provides a small opening unsuitable for the retrieval of intraruminal boluses at regular intervals. The bolus also cannot be suspended on a nylon thread as that may impede their natural gravitation to the reticulum. The serial slaughter of sheep, even if they are to be slaughtered for meat consumption is expensive and laborious as it requires large numbers of animals and is also restricted to certain times of the year.

The development work described in this section is with a 19 mm diameter bolus. This diameter was chosen as alternative boluses already produced for sheep (the magnesium alloy product and a glass trace element bolus) were of the same diameter.

The target animal for such a bolus product was quite specific unlike the 25 mm bolus where it was for use in all classes of cattle. The target was a 70 kg ewe carrying twin lambs, and the bolus target was to have a retention of 95% or greater and supply at least 50% of the daily allowance of cobalt and selenium and no more than 100% of copper allowance from 8 weeks before lambing to in excess of 8 weeks after. This gives a bolus profile of an expected life of at least 112 days and to supply no more than 14.4 mg copper and at least 0.26 mg cobalt and 0.24 mg selenium daily.

This section describes the development and testing of the 19 mm bolus with the aim of meeting these targets.

# SECTION 5

**Experiment 5.1.**
**Initial studies with a 19 mm diameter bolus suitable for administration to sheep.**

**Experiment 5.2.**
**The release rate and retention of a single 19 mm diameter bolus when administered to ewes.**

**Experiment 5.3.**
**The faecal copper concentrations of lambs when given either two 19 mm boluses or no supplement.**

**Experiment 5.4.**
**The release rate and retention of two 19 mm diameter boluses when administered to adult wether sheep and the effect on their liver, faecal and blood plasma copper concentrations.**

**Experiment 5.5.**
**The release rate and retention of two 19 mm diameter boluses given only a single coat when administered to lambs and the effect on their liver, faecal and blood plasma copper concentrations.**

**Experiment 5.6.**
**The release rate and retention of two 19 mm diameter boluses with a density of 3.0 g/cm$^3$ when administered to ewes.**

**Experiment 5.7.**
**The release rate and retention of a single 19 mm commercial prototype bolus when administered to ewes and lambs.**

## Experiment 5.1

## Initial studies with a 19 mm diameter bolus suitable for administration to sheep.

### Introduction

A 19 mm diameter die was produced to the same design as the 25 mm diameter die used to produce the boluses for cattle. This dimension was chosen as the bolus products already marketed for sheep were of that diameter and so the same sized oesophageal balling gun could be used. That diameter would seem to be appropriate for sheep over 20 kg liveweight. This experiment assesses the release rate of a 19 mm bolus firstly in fistulated cows. Thereafter they were administered to Blackface ewes which were to be slaughtered and this allowed the boluses to be recovered.

### Materials and methods

These were as in Section 1.5 using Matrix No 1, but with a 19 mm die. The boluses produced were of the following dimensions.

Weight 40 g; Length 5.2 cm; Density 2.5 g/cm$^3$

An example of the boluses used in this experiment and others within this section are shown in Plate 15.

Initially in Experiment 5.1A six boluses were tested in pairs in each of three fistulated cows. The test period was 28 days and the cows were given hay and concentrates for the whole of that period.

In Experiment 5.1B a further 56 boluses were administered singly to 18 ewes and in pairs to 19 Blackface ewes which were housed and given hay and concentrates. The ewes were slaughtered at either 11, 18 or 25 days after administration.

The reticulo-rumen of each sheep was thoroughly searched at slaughter and the boluses recovered and weighed. The weight loss was divided by the number of days since administration to give a mean daily release rate over that period.

**Plate 15.**
**Examples of 19 mm diameter boluses for administration to sheep. The bolus at the top is the commercial prototype from Experiment 5.7.**

## Results

### Experiment 5.1A

The mean release rate in fistulated cows is shown in Table 5.1. The boluses were found in the reticulum on each of the four occasions when they were removed for weighing and all were recovered at the end of the 28 day test period.

**Table 5.1 The mean release rate (g/bolus/day) of 19 mm diameter boluses when placed in pairs into each of three fistulated cows**

| Days | mean | s.d. |
|---|---|---|
| 0-7 | 0.42 | 0.319 |
| 8-14 | 0.23 | 0.068 |
| 15-21 | 0.19 | 0.077 |
| 22-28 | 0.16 | 0.050 |
| 0-28 | 0.25 | 0.093 |
| **Mean total weight loss (g)** | **7.1** | **2.61** |

Experiment 5.1A showed that the 19 mm diameter bolus gave a high release rate considering that the surface area is only half that of the 25 mm diameter bolus. The variation in release rate was also high. The c.v. for the mean release rate over days 0-28 was 36%. The release rate slowed progressively from 0.42 g/bolus/day during the first 7 days to 0.16 g/bolus/day during the period 22-28 days. The results were however promising enought to allow Experiment 5.1B with the ewes to be conducted.

**Experiment 5.1B**

The results for ewes given a single bolus are given in Table 5.2 and for those given a pair of boluses in Table 5.3.

When a single bolus was administered to the ewes the mean daily loss (g/bolus/day) declined from 0.26 g to 0.19 g from days 0-11 to days 11-18 respectively. The boluses appeared to continue to release material since the total loss to day 18 was 3.4 g and to day 25 was 4.6 g.

From the mean total weight loss data for days 11, 18 and 25 the daily release over days 0-11, days 12-18 and days 19-25 can be calculated to be 0.26, 0.07 and 0.17 g respectively, but this data should be considered with some reservation because of the small number of boluses recovered on day 25.

The retention of boluses was 80-86% at days 11 and 18 but was reduced to 50% by day 25. This level of retention is far too low for a bolus to be acceptable as a means of supplying trace elements to sheep. It is assumed that the boluses were lost due to regurgitation.

**Table 5.2 The mean release rate (g/bolus/day) of single 19 mm diameter boluses when administered to ewes and recovered at slaughter 11, 18 or 25 days after administration.**

| **Days** | **11** | **18** | **25** | | | |
|---|---|---|---|---|---|---|
| No. of sheep | 5 | 7 | 6 | | | |
| | 0.16 | 0.17 | 0.24 | | | |
| | 0.24 | 0.15 | 0.15 | | | |
| | 0.33 | 0.19 | 0.16 | | | |
| | 0.31 | 0.18 | N.R. | | | |
| | N.R. | 0.24 | N.R. | | | |
| | | 0.22 | N.R. | | | |
| | | N.R. | | | | |
| loss | | | | Significance of mean daily | | |
| | | | | | SED | |
| Sig P | | | | | | |
| **Mean**[+] | **0.26** | **0.19** | **0.18** | **11 v 18 days** | **0.036** | **NS** |
| **s.d.** | 0.076 | 0.035 | 0.049 | 11 v 25 days | 0.024 | 0.05 |
| **Retention %** | **80** | **86** | **50** | | | |
| **Mean total weight loss (g)** | **2.9** | **3.4** | **4.6** | | | |

N.R. not recovered from reticulorumen at slaughter

[+] Mean for only those boluses recovered.

For ewes given two boluses the situation is complicated by an even poorer overall retention of about 65% (Table 5.3). Of the original 38 boluses administered to these ewes 13 were not recovered; four ewes lost both boluses and six ewes each lost one of the pair. In contrast to the single bolus the best retention rate was from that observed at day 25.

**Table 5.3 The mean release rate (g/bolus/day) of a pair of 19 mm diameter boluses when administered to ewes and recovered at slaughter, 11, 18 or 25 days after administration.**

| **Days** | **11** | | **18** | | **25** | |
|---|---|---|---|---|---|---|
| No of sheep | 5 | | 6 | | 8 | |
| | 1.30 | 1.09 | 0.70 | 0.71 | 0.59 | 0.54 |
| | 0.68 | 0.72 | 0.39 | 0.47 | 0.47 | 0.53 |
| | 0.33 | N.R. | 0.45 | N.R. | 0.16 | 0.13 |
| | 0.59 | N.R. | 0.57 | N.R. | 0.44 | 0.39 |
| | N.R. | N.R. | N.R. | N.R. | 0.30 | 0.14 |
| | | | N.R. | N.R. | 0.20 | 0.44 |
| | | | | | N.R. | 0.47 |
| | | | | | N.R. | |
| | | | | | N.R. | |
| **Complete pair** | | | | | | |
| **Mean** | **0.95** | | **0.57** | | **0.38** | |
| **s.d.** | 0.299 | | 0.161 | | 0.163 | |
| **Incomplete pair** | | | | | | |
| **Mean** | **0.46** | | **0.47** | | **0.20** | |
| **s.d.** | 0.180 | | 0.096 | | - | |
| **Retention %** | **60** | | **50** | | **81** | |
| **Mean total weight loss (g) per bolus** | **10.5** | | **10.2** | | **9.6** | |

N.R. not recovered from reticulo-rumen at slaughter.

The release rate for complete pairs of boluses was 0.95 g/bolus/day at day 11 but declined to 0.38 g at day 25. This was approximately three times greater than for the single bolus (Table 5.2) initially but declined to a factor of two by day 25. Two boluses thus provide a supply to the animal some 4-6 times greater than that from a single bolus.

For ewes initially given two boluses but where only an incomplete pair were recovered the release rate at days 11 and 18 was also greater than that from a

single bolus suggesting that the missing bolus was present for a time before it was lost, presumably by regurgitation. On day 25 there was only one such bolus and its release rate (0.20 g/bolus/day) was similar to those from sheep given a single bolus (0.18 g/bolus/day) perhaps indicating very early loss of the other bolus.

The total weight loss for boluses given in pairs over days 0-25 and 0-18 was less than that over days 0-11. This is an indication that the boluses may have stopped releasing material after day 11. The poor retention of the boluses however makes it difficult to draw any conclusions.

**Conclusion**

The mean total weight loss per bolus when two boluses were given to fistulated cows was 7.1 g after 28 days and when given to ewes 9.6 g after 25 days. The rate of release in ewes may perhaps be expected to be faster due to a smaller reticulum leading to increased contact between the boluses. Bearing this is mind the fistulated cows do provide a reasonable model to test the release rate of 19 mm boluses although they do not give any indication of the extent to which boluses may not be retained by sheep and lost because of regurgitation. It should be recognised that the density of these boluses was only 2.5 $g/cm^3$.

The release of material from two boluses was initially too high (0.95 g/bolus/day) and appeared to stop at around 11 days. The recovery rate of 65% was also very poor.

A single bolus continued to release material to at least 25 days and with a mean release rate/day over that period of 0.21 g would supply 41 mg copper, 0.1 mg cobalt and 0.1 mg selenium. For ewes the bolus would thus supply three times the requirement (12.5 mg/day) of copper and almost half the requirement for cobalt and selenium. Prolonged supplementation of copper at that high level of copper would have the risk of problems with toxicity. Considering these problems it was decided to test further a single bolus to see if the release rate could be reduced to an acceptable level. e.g. A release rate of 0.06 g/day would supply 12 mg copper/day. At the same time it would be desirable to increase the density to improve retention.

**Experiment 5.2.**

**The release rate and retention of a single 19 mm diameter bolus when administered to ewes.**

## Introduction

Following the results of Experiment 5.1B it was decided to monitor the release and retention of a single bolus over a longer period.

## Materials and methods

These were as in Experiment 5.1B using a 40 g bolus with a density of 2.5 g/cm$^3$. One of these boluses was administered to each of 35 Blackface ewes. The ewes were housed and given hay and concentrates. They were slaughtered in three groups at 45, 59 and 66 days after bolus administration. At slaughter the reticulo-rumens were searched and the boluses recovered and weighed. The total weight loss was divided by the number of days since administration to give the mean daily release rate over that period.

## Results

These are shown in Table 5.4. The boluses showed no coating but the exposed surface was darker in colour than seen in cattle-sized boluses after similar periods in the rumen. The overall mean retention from all three slaughter dates was 77% and was similar to that observed in Experiment 5.1B when a single bolus was given. From this we might assume that little or no regurgitation occurs after day 25.

The mean total bolus weight loss for all three dates was less than that at day 25 found earlier in Experiment 5.1B perhaps indicating that there is no loss of material after that time. In this way the single bolus performs in a similar manner to the pairs of boluses in Experiment 5.1A except that the slowing down of release rate takes slightly longer, but occurs at a lower point of total weight loss. Why this slowing down occurs is unclear but it may be a function of the smaller diameter used since in Experiment 5.1A the release rate of three pairs of the boluses in fistulated cows showed a similar trend generally not seen when testing 25 mm diameter boluses in these animals.

**Table 5.4** **The mean release rate (g/bolus/day) of a single 19 mm diameter bolus when administered to ewes and recovered at slaughter 45, 59 or 66 days after administration.**

| **Days** | **45** | **59** | **66** |
|---|---|---|---|
| No of sheep | 12 | 10 | 13 |
| | 0.05 | 0.04 | 0.06 |
| | 0.13 | 0.10 | 0.04 |
| | 0.07 | 0.07 | 0.06 |
| | 0.07 | 0.11 | 0.07 |
| | 0.11 | 0.09 | 0.04 |
| | 0.05 | 0.04 | 0.04 |
| | 0.06 | 0.05 | 0.09 |
| | 0.06 | N.R. | 0.07 |
| | 0.05 | N.R. | 0.09 |
| | 0.07 | N.R. | 0.04 |
| | N.R. | | N.R. |
| | N.R. | | N.R. |
| | | | N.R. |
| **Mean** | **0.07** | **0.07** | **0.06** |
| **s.d.** | 0.028 | 0.028 | 0.019 |
| **Retention %** | **83** | **70** | **77** |
| **Mean total weight loss (g)** | **3.2** | **4.3** | **3.9** |

N.R. not recovered from reticulo-rumen at slaughter.

There were no significant differences in the mean bolus weight loss over different periods of time.

**Experiment 5.3.**

**The faecal copper concentrations of lambs when given either two 19 mm boluses or no supplement.**

## Introduction

Experiments 5.1 and 5.2 demonstrated that the 19 mm boluses were poorly retained by sheep and that the boluses may have ceased releasing material after as little as 11 days. To investigate these problems further it was decided to attempt to trace the release of copper from the bolus by monitoring faecal copper concentrations. This method has been used with some success with other bolus technologies (Ellis, Shallow & Judson, 1987; Khandaker & Telfer, 1988)

For most diets the availability of copper to sheep is less than 0.04 and most of the unabsorbed copper is excreted in the faeces (Mertz, 1987). With a bolus containing 19.25% copper the administration and subsequent release of material from the bolus should increase faecal copper concentrations considerably. If an estimate of total faecal copper due to the bolus can be made then the release rate of the bolus or boluses may be estimated.

It was hoped that the use of this method would allow determination of the retention and release rate of the boluses.

## Materials and methods

Eight Suffolk cross lambs, of mean liveweight 36 kg were housed in slatted individual pens and fed 1 kg of grass nuts/day divided into two feeds. The mean copper concentration in the feed was 30 mg/kg DM.

Four of the lambs were given two 19 mm boluses administered using an oesophageal balling gun. The boluses were composed of bolus Matrix No 1. Their dimensions were; weight 40 g, length 5.2 cm, density 2.5 g/cm$^3$. They were produced by the method as detailed in Section 1.5. Two boluses were given since the problems outlined were considerably greater when boluses were administered as pairs. The remaining four lambs were not bolused and acted as a control.

Faecal grab samples (taken directly from the rectum) were obtained prior to bolus administration and on the first and second day after bolusing. Thereafter samples were taken every 4 days until day 58. Faecal grab samples were taken at 10.00 h, and the dry weight of these was typically 3-5 g. Once dried the faeces

were analysed for copper. To calculate total copper output due to the boluses, the faecal DM output for the lambs on this diet was required to be determined. For this the control sheep were housed in metabolism cages from days 7-14. This allowed a total faecal collection to be obtained.

**Results**

The mean dry matter faecal output as determined by the four control lambs when housed in metabolism cages was 0.38 kg/day (s.d. 0.017).

Table 5.5 gives the mean faecal copper concentrations for the bolused and control lambs, this data is also shown in Figure 5.1. For the control lambs the mean faecal Cu concentrations ranged from 58-73 mg/kg DM and on each sampling variation between lambs was generally low, c.v. 2.9-9.4%.

**Table 5.5 Mean faecal copper concentrations (mg/kg DM) of the four lambs given two 19 mm diameter boluses and four lambs used as a control.**

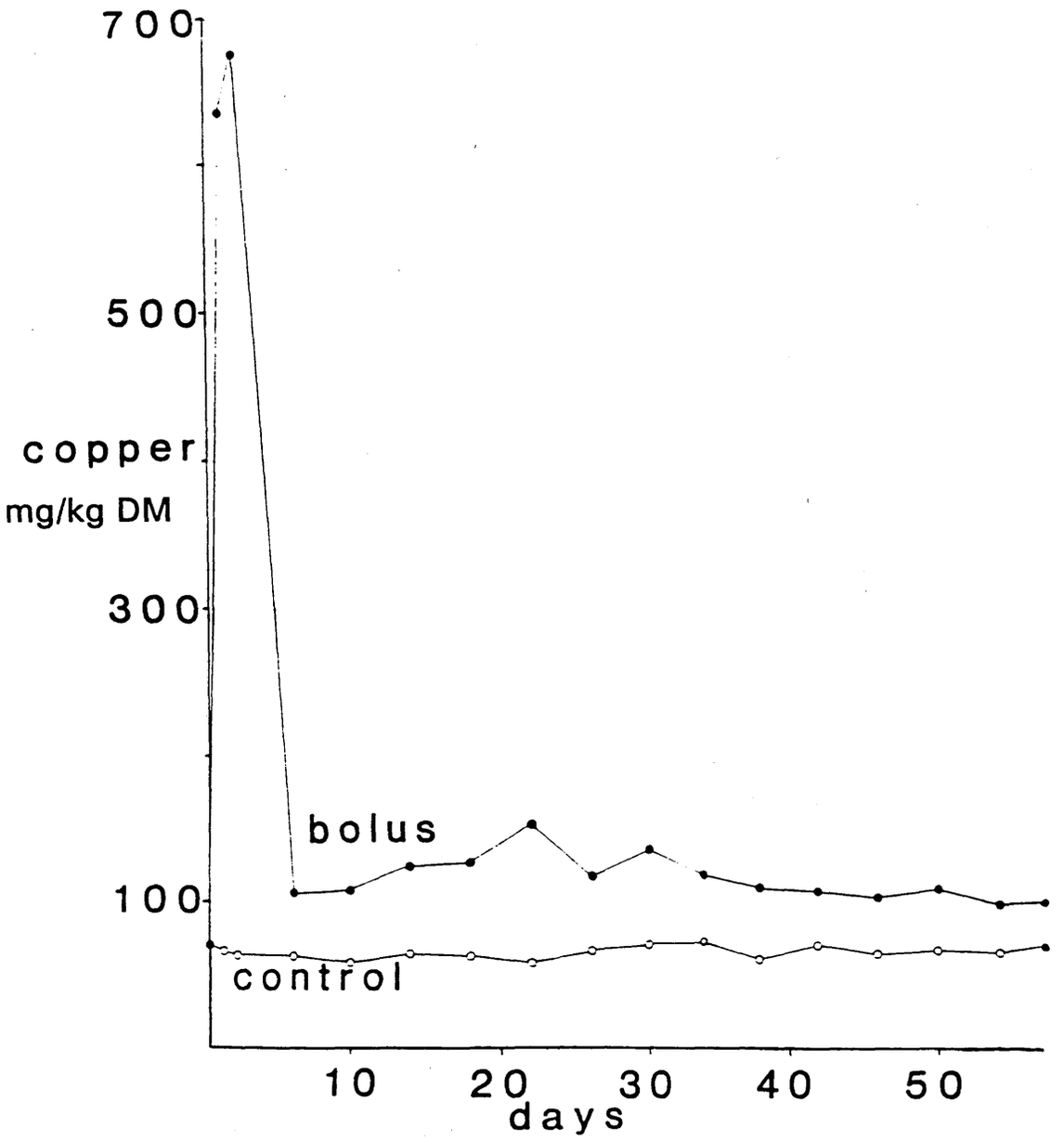
| | Bolused | | Control | | | |
|---|---|---|---|---|---|---|
| Days | mean | s.d. | mean | s.d. | SED | Sig P |
| **0** | 70 | 6.0 | 67 | 3.9 | 4.9 | NS |
| **1** | 638 | 130.0 | 67 | 4.2 | 65.0 | .001 |
| **2** | 677 | 272.1 | 64 | 5.8 | 136.0 | .01 |
| **6** | 106 | 15.2 | 63 | 2.4 | 8.1 | .01 |
| **10** | 108 | 27.0 | 59 | 1.7 | 13.5 | .01 |
| **14** | 125 | 25.9 | 65 | 3.0 | 13.1 | .01 |
| **18** | 127 | 33.1 | 63 | 3.0 | 16.6 | .01 |
| **22** | 152 | 40.8 | 58 | 2.9 | 20.5 | .01 |
| **26** | 117 | 26.2 | 66 | 3.2 | 13.2 | .01 |
| **30** | 135 | 39.0 | 71 | 6.7 | 19.8 | .05 |
| **34** | 118 | 10.5 | 72 | 6.0 | 6.1 | .001 |
| **38** | 109 | 14.4 | 60 | 3.0 | 7.4 | .01 |
| **42** | 107 | 11.0 | 70 | 2.1 | 5.6 | .01 |
| **46** | 103 | 2.7 | 64 | 2.4 | 1.8 | .001 |
| **50** | 109 | 18.7 | 67 | 3.2 | 9.5 | .01 |
| **54** | 99 | 15.4 | 66 | 2.9 | 7.8 | .01 |
| **58** | 102 | 16.1 | 73 | 6.5 | 8.7 | .05 |

The administration of two 19 mm boluses increased the faecal copper concentrations of the bolused lambs. The variation between lambs at sampling occasions was however large. The c.v. ranged from 2.6 to 41%, however most of the larger coefficients of variation were recorded over the first few samples.

The difference between the mean concentrations of bolused and control lambs was significantly different at all sampling occasions (P generally $<0.01$). On days 1 and 2 after bolusing the mean faecal Cu concentrations of the bolused lambs were 638 and 677 mg/kg DM that is around 10 times greater than the control concentrations. These were reduced to 132 mg/kg DM by day 6 and were between 100 and 150 mg/kg DM at all samplings until day 58. This suggested that from day 6 to day 58 the two boluses were releasing at a fairly constant rate. There were also no indications that regurgitation had occurred. An estimate of the total faecal copper can be made using the faecal dry matter output. The total faecal copper due to the boluses (i.e. faecal DM output x difference in faecal Cu between bolused and control lambs) on days 2 and 58 is thus 232 and 11 mg respectively. Since the bolus matrix contains 19.25% copper this gives an estimated bolus release rate of 0.603 g and 0.029 g Cu/bolus/day respectively.

If the probable weight loss of each bolus by day 11 is calculated using this model the value is 9.5 g. The recorded figure in Experiment 5.1B for boluses in ewes was 10.5 g. Hence this method is reasonably accurate. However further evaluation in sheep which are due for slaughter would allow better comparison of the method with actual release rates.

**Figure 5.1.**
**Mean faecal copper concentrations (mg/kg DM) of the four lambs given two 19 mm diameter boluses and four lambs used as a control over a period of 58 days.**


700
500
300
100
copper
mg/kg DM
bolus
control
10
20
30
40
50
days

## Experiment 5.4.

**The release rate and retention of two 19 mm diameter boluses when administered to adult wether sheep and the effect on their liver, faecal and blood plasma copper concentrations.**

### Introduction

Following the encouraging results of Experiment 5.3. this experiment was designed to compare faecal copper monitoring with the actual release rate of boluses recovered from sheep at slaughter. The boluses used had a density of 3.0 g/cm$^3$ achieved by the addition of iron shot to the base of the bolus.

### Materials and methods

Twelve Suffolk cross wether sheep of mean initial liveweight 69 kg were individually penned on slats and fed 1.5 kg of a fibre-containing concentrate per day divided into two feeds. This ration contained 12 mg Cu/kg DM.

Six of the sheep were each given two 19 mm diameter boluses using an oesophageal balling gun. The boluses weighed 24 g and were composed of 20 g of bolus matrix with 4 g of iron shot added to increase the density, their dimensions being; weight 24 g, length 2.8 cm, density 3.0 g/cm$^3$. The boluses were produced as detailed in Section 1.5. The remaining six sheep were not bolused and acted as a control.

Blood and faecal grab samples were taken at the same time as the boluses were given and were repeated at intervals throughout the 30 days until the sheep were slaughtered. The grab samples were taken directly from the rectum at 10.00 h on each occasion.

At slaughter a sample of liver was obtained from all the sheep and the reticulo-rumen of the bolused sheep were searched and the boluses recovered.

Plasma, faecal and liver samples were assayed for copper.

## Results

### Bolus recovery and release rate

All twelve boluses were found at slaughter giving a retention of 100%. The release rates for the boluses are given in Table 5.6.

The total weight loss was only 1.4 g giving a release rate over days 0-30 of 0.5 g/bolus/day. This release rate is much less than that shown in Experiment 5.1B and that estimated in Experiment 5.3. This may be due to the shorter length of the boluses used in this present experiment (2.8 cm v 5.2 cm) leading to less interaction and therefore reduced mutual erosion.

### Liver copper concentrations

These are also shown in Table 5.6. Both bolused and control sheep had liver copper levels well above the threshold of about 30 mg/kg DM associated with deficiency. This was expected since the sheep had been housed for the previous eighteen months and had received normal hay and concentrate diets. The elevated liver copper levels of the control sheep and the low release rate of the bolus resulted in there being no significant difference between the liver copper concentrations of the bolused and control sheep.

### Faecal copper

The mean faecal copper concentrations are shown in Table 5.7. The sheep not given boluses had a mean faecal Cu concentration over the period of the experiment which varied between 21-29 mg/kg DM.

The bolused sheep had much higher concentrations on days 1 and 2 but day 6 was the last day when there was a significant difference between the bolused and control sheep. On that day the difference was 12 mg/kg DM. If the DM intake was 1.4 kg/day and digestibility is assumed as 0.6 then the faecal DM output is 0.56 kg and the difference in total faecal copper was 7 mg. If that originated from the two boluses then the mean weight loss of each bolus was only 0.013 g/bolus/day. On day 1 however it was about 0.27 g/bolus/day.

From the faecal copper results it would appear that the boluses ceased to release material about 10 days after administration. If the release rates for days 1 and 2 are calculated and it is assumed that the bolus release rate declines linearly to zero at day 10 then the total loss of material over the 30 days is 1.35 g/bolus. The mean total loss of the twelve boluses recovered from the sheep was 1.4 g. The faecal copper concentrations therefore give a reasonable estimate of the bolus release.

**Table 5.6 The initial and recovered weights (g) of the boluses and the liver copper concentrations at slaughter of bolused and control sheep.**

| Sheep | Bolus weight Initial | Recovered | Release rate g/day d 0-30 | Liver copper mg/kg DM |
|---|---|---|---|---|
| **1** | 24.3 | 23.4 | 0.03 | 340 |
| | 24.3 | 23.2 | 0.04 | |
| **2** | 24.3 | 22.9 | 0.05 | 218 |
| | 24.3 | 23.3 | 0.03 | |
| **3** | 24.2 | 22.9 | 0.04 | 227 |
| | 24.2 | 23.0 | 0.04 | |
| **4** | 24.3 | 22.8 | 0.05 | 192 |
| | 24.2 | 22.5 | 0.06 | |
| **5** | 24.2 | 22.3 | 0.06 | 143 |
| | 24.2 | 22.5 | 0.06 | |
| **6** | 24.3 | 23.0 | 0.04 | 193 |
| | 24.2 | 22.9 | 0.04 | |
| **mean** | **24.3** | **22.9** | **0.05** | **219** |
| **s.d.** | 0.05 | 0.33 | 0.011 | 66.2 |
| Controls, no boluses given | | | | |
| **7** | | | | 72 |
| **8** | | | | 236 |
| **9** | | | | 185 |
| **10** | | | | 158 |
| **11** | | | | 174 |
| **12** | | | | 97 |
| **mean** | | | | **154** |
| **s.d.** | | | | 60.0 |

Mean liver copper SED = 36.5 NS

**Table 5.7 Mean faecal copper concentration (mg/kg DM) of six wethers given two 19 mm diameter boluses and six wethers used as a control.**

| | Bolused | | Control | |
|---|---|---|---|---|
| **Day** | mean | s.d. | mean | s.d. |
| **0** | 23 | 0.8 | 22 | 0.4 |
| **1** | 204 | 18.1 | 21 | 0.4 |
| **2** | 151 | 19.0 | 25 | 1.2 |
| **6** | 37 | 4.1 | 25 | 1.5 |
| **10** | 26 | 1.4 | 25 | 0.5 |
| **14** | 29 | 0.9 | 29 | 1.1 |
| **18** | 26 | 1.3 | 24 | 0.8 |
| **22** | 30 | 3.0 | 26 | 0.6 |
| **26** | 25 | 0.9 | 24 | 0.7 |
| **30** | 25 | 0.8 | 25 | 0.9 |

**Plasma copper**

Throughout the experiment the blood copper concentrations of all the sheep, bolused and control remained within the normal range.

**Conclusions**

The comparison of faecal copper concentrations of bolused and control sheep gave an estimate of bolus weight loss which is similar to the actual weight loss recorded at slaughter. The boluses had a density of $3g/cm^3$ and retention was 100%. In this experiment the boluses had a low release rate which might possibly be explained by the shorter length of the boluses relative to those used in Experiments 5.1, 5.2 and 5.3.

## Experiment 5.5.

**The release rate and retention of two 19 mm diameter boluses given only a single coat of polymer resin when administered to lambs and the effect on the liver, faecal and blood plasma copper concentrations.**

### Introduction

Experiment 5.4. showed that monitoring faecal copper levels could give a reasonable estimate of bolus weight loss. However in that experiment the bolus release rate was very low at only 0.045 g/day. This present experiment was designed to further confirm the value of faecal copper monitoring. In an attempt to increase the release rate of the bolus only a single coat of resin was applied. The results of Experiment 2.7 had shown that applying only a single coat of resin would give an increase in bolus release rate of 115% over boluses given the standard two coats.

### Materials and methods

Eight Suffolk cross wether lambs of mean initial liveweight 36 kg were housed in a straw bedded pen and group fed 750 g of a barley-soya concentrate and 350 g of hay. Both hay and concentrate portions were given over two feeds. The total diet contained 7 mg/kg DM copper.

Four of the lambs each received two 19 mm diameter boluses administered by means of an oesophageal balling gun. The boluses weighed 24 g and were composed of 20 g of Matrix No 1 plus 4 g iron shot. Their dimensions were, weight 24 g, length 2.8 cm, density 3.0 g/cm$^3$. The only difference to those given in Experiment 5.4. was that only a single coat of resin was applied. The boluses were produced as detailed in Section 1.5. The remaining four lambs were not bolused and acted as a control.

Blood and faecal grab samples were taken prior to bolus administration and were repeated at 7 day intervals until day 35 when the lambs were slaughtered. Blood and faecal samples were taken at 10.00 h on each occasion.

At slaughter the reticulo-rumen of each of the bolused lambs was searched and the boluses recovered and weighed. A sample of liver was obtained from all the lambs. Blood plasma, faecal grab samples and liver samples were analysed for copper.

## Results

### Bolus recovery and release rate

All lambs were slaughtered 35 days after bolus administration. The final weight of each bolus is shown in Table 5.8. Seven of the eight boluses administered were recovered. The mean release rate over days 0-35 was 0.24 g/day for all boluses and 0.25 g/day for boluses which were retained as a pair until slaughter. The single bolus which was recovered from lamb No 3 had a release rate of 0.17 g/day. This lower rate was probably due to the absence of mutual erosion.

The release rate of almost 0.5 g/day from two boluses supplies an excessive 92 mg/day of copper over the 35 day period.

**Table 5.8 The initial and recovered weights of the boluses and the liver copper concentrations at slaughter of bolused and control lambs.**

| | Bolus weight (g) | | Release rate g/day d 0-35 | Liver copper mg/kg DM |
|---|---|---|---|---|
| **Lamb No** | Initial | Recovered | | |
| **1** | 24.0 | 15.0 | 0.26 | 648 |
| | 23.9 | 16.7 | 0.21 | |
| **2** | 23.8 | 13.4 | 0.30 | 756 |
| | 24.1 | 14.4 | 0.28 | |
| **3** | 24.1 | 18.3 | 0.17 | 503 |
| | 23.5 | N.R. | - | |
| **4** | 23.6 | 15.2 | 0.24 | 829 |
| | 23.9 | 16.8 | 0.20 | |
| **mean** | **23.9** | **15.9** | **0.24** | **684** |
| **s.d.** | 0.22 | 1.72 | 0.046 | 141.7 |
| Controls, no boluses given | | | | |
| **5** | | | | 91 |
| **6** | | | | 76 |
| **7** | | | | 190 |
| **8** | | | | 122 |
| **mean** | | | | **120** |
| **s.d.** | | | | 50.6 |

N.R. - not recovered from the reticulo-rumen at slaughter.

Mean liver copper SED 75.3 $P<0.001$.

**Liver copper concentrations**

This excessive supply of copper from the bolus was reflected in the liver copper levels seen at slaughter (also in Table 5.8). The mean concentration for the bolused lambs was 684 mg/kg DM whereas the control lambs had a mean concentration of 120 mg/kg DM. This increased liver copper status occurred over a short period. It shows that copper oxide powder is effective in increasing the hepatic copper store of lambs. However the continued supply of copper to these lambs in excess to their requirement might eventually have led to toxicity.

It is interesting to note that lamb No 3 which lost one bolus had the lowest liver copper concentration of the bolused sheep but even it was in excess of 500 mg/kg DM.

**Faecal copper**

The mean faecal copper concentrations (mg/kg DM) for the boluses and control sheep are shown in Table 5.9. Administration of two 19 mm diameter boluses markedly increased the faecal copper concentrations of the lambs over the 35 day period of the experiment. Control lambs had mean concentrations ranging between 16 and 31 mg/kg DM whereas at day 7 bolused lambs had a mean concentration of 265 mg/kg DM and even at day 35 the mean concentration was still 174 mg/kg DM. The faecal copper concentrations of lamb No 3 fell after day 14 until it was within the range of concentration shown by control lambs. For example at day 28 the faecal copper concentration of that lamb was only 17 mg/kg DM and the mean value for control lambs was 16 mg/kg DM.

The results for the other three bolused lambs show that the boluses were still releasing material at day 35. The difference in mean faecal copper concentrations for the two groups on that day was 158 mg Cu/kg DM. With a DM intake of 0.95 kg and an assumed digestibility coefficient of 0.50 then the faecal DM output was 0.48 kg/day. On this basis the mean total faecal copper output on day 35 due to the boluses was 76 mg. This equates to 0.20 g/bolus/day as the bolus contained 19.25% copper. The estimated release rates at days 7, 14, 21, 28 and 35 were therefore 0.30, 0.18, 0.20, 0.11, 0.20 g/bolus/day. Although these estimates do not give any figure for the initial surge in release rate they do agree reasonably well with the recorded release rate at slaughter of 0.25 g/bolus/day over 0-35 days.

**Table 5.9** **Mean faecal copper concentrations (mg/kg DM) of four lambs given two 19 mm diameter boluses and four lambs used as a control.**

| | Bolused | | Control | | | |
|---|---|---|---|---|---|---|
| **Day** | mean | s.d. | mean | s.d. | SED | Sig P |
| **0** | 37 | 6.5 | 31 | 2.7 | 3.6 | NS |
| **7** | 265 | 184.4 | 27 | 3.0 | 142.1 | NS |
| **14** | 176 | 81.3 | 33 | 2.7 | 40.6 | 0.05 |
| **21*** | 181 | 42.8 | 23 | 2.7 | 21.4 | 0.001 |
| **28** | 106 | 16.8 | 16 | 2.4 | 8.5 | 0.001 |
| **35** | 174 | 91.0 | 16 | 4.3 | 45.6 | 0.05 |

* From day 21 onwards one lamb had a low faecal copper concentration. It was presumed that the boluses had been regurgitated and it was excluded from the mean data from day 21 onwards.

**Plasma copper**

The plasma copper concentrations of the lambs over the period of the experiment are shown in Table 5.10. Adequate values are within the range 9.4 - 24.0 umol/litre. The bolused lambs maintained values within that range throughout the experiment. In contrast the control lambs had plasma values which fell gradually over the period of the experiment such that they were deficient by day 28-35.

**Table 5.10 Mean and individual plasma copper concentrations (umol/litre) for four lambs given two 19 mm diameter boluses and for four lambs used as a control.**

| **Days** | **0** | **7** | **14** | **21** | **28** | **35** |
|---|---|---|---|---|---|---|
| **Lamb No.** | | | | | | |
| BOLUSED | | | | | | |
| **1** | 10.5 | 15.7 | 12.6 | 12.1 | 16.8 | 12.9 |
| **2** | 10.1 | 14.3 | 15.9 | 13.4 | 10.7 | 10.9 |
| **3** | 13.7 | 13.9 | 13.4 | 13.9 | 13.2 | 13.2 |
| **4** | 7.2 | 15.3 | 14.3 | 11.8 | 13.5 | 19.0 |
| **mean** | **10.4** | **14.8** | **14.1** | **12.8** | **13.6** | **14.0** |
| **s.d.** | 2.66 | 0.84 | 1.42 | 1.01 | 2.50 | 3.49 |
| CONTROL | | | | | | |
| **5** | 12.7 | 11.3 | 12.0 | 10.9 | 8.5 | 9.8 |
| **6** | 6.5 | 9.3 | 7.6 | 5.5 | 5.4 | 5.5 |
| **7** | 10.9 | 14.3 | 12.1 | 10.5 | 9.4 | 9.2 |
| **8** | 8.7 | 10.1 | 10.7 | 9.9 | 9.1 | 9.6 |
| **mean** | **9.7** | **11.3** | **10.6** | **9.2** | **8.1** | **8.5** |
| **s.d.** | 2.69 | 2.19 | 2.10 | 2.50 | 1.84 | 2.03 |
| **SED** | 1.90 | 1.17 | 1.27 | 1.35 | 1.55 | 1.98 |
| **Sig P** | NS | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |

**Discussion**

The plasma copper results show that the boluses supply copper in a sufficiently available form to prevent lambs from becoming hypocupraemic. Similarly the large increases in hepatic copper reserves indicate its effectiveness as a copper supplement to sheep. The bolus release rate recorded at slaughter and estimated by faecal copper levels was about five times greater than that of similar

boluses in Experiment 5.4. where the boluses received two coats of resin. With 25 mm diameter boluses in fistulated cows single coat boluses released at twice the rate of the normal two coated boluses (Experiment 2.7)

The use of faecal copper concentrations to estimate bolus release rates provided some interesting results. It detected the loss of a bolus from lamb No 3 and from the results thereafter it showed that without the second bolus the release of material from the first either ceased or continued at a very slow rate. The prediction of release rates using faecal copper gives similar figures to that obtained from slaughter, especially if the peak in release over the first two days shown in Experiments 5.3. and 5.4. also occurred with these boluses. Even by day 35 however the boluses were still supplying 76 mg/copper/day when the daily allowance even for adult sheep is only 6 mg/kg DM (MAFF et al 1983).

A two bolus administration where only a single coat of resin is applied does not appear to be a useful product for sheep.

**Experiment 5.6.**

**The release rate and retention of two 19 mm diameter boluses with a density of 3.0 g/cm$^3$ when administered to ewes.**

## Introduction

In Experiment 5.1B the retention of two 19 mm diameter boluses when administered to ewes was only 65%. A bolus with a density of 3.0 g/cm$^3$ had been produced and tested in Experiments 5.4. and 5.5. with a retention of 95% over both experiments. The release rate of the bolus had been low in Experiment 5.4. but when the coating was reduced to a single coat in Experiment 5.5. this had increased dramatically.

This experiment used Blackface ewes given two boluses each given two coats of resin. Apart from the diet this experiment was then similar to Experiment 5.1B but with the new increased density bolus.

## Materials and methods

Fifty-two Blackface ewes were out-wintered at grass and additionally given 0.25 kg/head/day of sugar beet pulp nuts. Twenty-six ewes were given two 19 mm boluses at the start of the trial. These were administered using an oesophageal balling gun. The boluses weighed 24 g and were composed of 20 g of bolus Matrix No 1 and 4 g of iron shot placed in the bottom of the die before pressing. The inclusion of the iron shot gave the bolus a density of 3.0 g/cm$^3$. The boluses were otherwise produced as detailed in Section 1.5. Their dimensions were; weight 24 g, length 2.8 cm, density 3.0 g/cm$^3$. The remaining ewes were not bolused and acted as a control.

The ewes were slaughtered in three batches at 63, 76 and 98 days after bolus administration. At slaughter the reticulo-rumen of the bolused ewes was searched thoroughly and the boluses recovered and weighed. A sample of liver was also taken from both bolused and control ewes.

## Results

### Bolus recovery and release rate

Overall retention of the boluses was 88% with four ewes apparently losing a single bolus and one ewe losing both boluses. This was a reasonable improvement from Experiment 5.1B where the bolus density was only 2.5 g/cm$^3$ and retention only 65% The result is however somewhat less than the 95% retention aimed for and recorded in Experiments 5.4. and 5.5. Trials using more sheep would be required to establish a better picture.

Eight days after the boluses were administered one ewe died. Post mortem examination revealed that this was due to one of the boluses penetrating the oesophagus and lodging in the trachea.

The mean release rates of the boluses on days 63, 76 and 98 were 0.09, 0.08 and 0.06 g/bolus/day (figures for individual boluses are shown in Table 5.11). The mean total weight losses per bolus were 5.67, 6.23 and 6.23 g respectively. Thus the release rate between days 63 and 76 was 0.043 g/bolus/day and from days 76 to 98 there was no apparent loss of material from the two boluses. These boluses had a much lower release rate than those in Experiment 5.1B where the total weight loss over 11 days was 10.5 g compared to the 6.23 g over 76 days in this experiment. Further work may be required to identify if this was due to dietary factors or to the shorter length of the boluses resulting in less abrasive action.

**Table 5.11 The mean release rate (g/bolus/day) of two 19 mm diameter boluses when given to ewes and recovered at slaughter 63, 76 or 98 days after administration.**

| **Days** | **63** | | **76** | | **98** | |
|---|---|---|---|---|---|---|
| No of sheep | 8 | | 8 | | 9 | |
| | 0.08 | 0.06 | 0.09 | 0.07 | 0.06 | 0.09 |
| | 0.10 | 0.12 | 0.08 | 0.08 | 0.04 | 0.05 |
| | 0.09 | 0.09 | 0.08 | 0.07 | 0.09 | 0.09 |
| | 0.08 | 0.09 | 0.08 | 0.09 | 0.07 | 0.07 |
| | 0.08 | 0.08 | 0.09 | 0.08 | 0.06 | 0.06 |
| | 0.07 | 0.09 | 0.07 | 0.08 | 0.09 | 0.06 |
| | 0.15 | N.R. | 0.12 | N.R. | 0.06 | 0.05 |
| | N.R. | N.R. | 0.07 | N.R. | 0.05 | 0.04 |
| | | | | | 0.06 | N.R. |
| **Mean** | **0.09** | | **0.08** | | **0.06** | |
| **s.d.** | 0.022 | | 0.014 | | 0.017 | |
| **Retention %** | **81** | | **88** | | **94** | |
| **Mean total weight loss (g)** | 5.67 | | 6.23 | | 6.23 | |

N.R. - not recovered from reticulo-rumen at slaughter.

**Liver copper**

The liver copper concentrations of the ewes at slaughter are given in Table 5.12. The twenty-five control ewes had a mean liver copper concentration of 22 mg/kg DM and since liver copper concentrations of less than 30 mg/kg DM are indicative of copper deficiency 85% of these ewes would be classified as deficient.

The twenty ewes which retained both boluses to slaughter had a mean liver copper concentration of 165 mg/kg DM. At all three slaughter dates the bolused ewes had a significantly greater ($P < 0.05$) liver copper concentration than those in the control group.

The five ewes which lost one or both of their boluses prior to slaughter had a mean lower copper concentration of 30 mg/kg DM and were therefore similar to the concentrations shown by the untreated control sheep. This is perhaps surprising since the release of material even from a single bolus remaining should have supplied sufficient copper to elevate the liver status. It may however be explained by unknown differences in initial copper concentrations in the liver, the weight of the liver and variations in supplementary feed intake and therefore in dietary copper intake.

**Table 5.12 Liver copper concentrations (mg/kg DM) at slaughter of bolused and control ewes 63, 76 or 98 days after bolus administration.**

| Day | 63 | | 76 | | 98 | |
|---|---|---|---|---|---|---|
| | Bolused | Control | Bolused | Control | Bolused | Control |
| | 165 | 19 | 62 | 19 | 121 | 39 |
| | 389 | 24 | 62 | 11 | 143 | 36 |
| | 363 | 12 | 50 | 7 | 283 | 18 |
| | 261 | 11 | 159 | 24 | 103 | 24 |
| | 252 | 21 | 228 | 13 | 162 | 36 |
| | 112 | 18 | *23 | 18 | 145 | 24 |
| | *38 | 16 | 60 | 14 | 75 | 27 |
| | *35 | 12 | *30 | 29 | 106 | 16 |
| | | 8 | | | *25 | |
| | | 64 | | | | |
| **Mean** | **257** | **21** | **104** | **17** | **142** | **28** |
| **s.d.** | 107.9 | 16.1 | 73.2 | 7.2 | 63.3 | 8.7 |
| **SED** | 44.3 | | 30.1 | | 22.2 | |
| **Sig P** | 0.01 | | 0.05 | | 0.01 | |

* Either both or one of the boluses was not recovered from these ewes and their liver copper values have been excluded from the mean values given.

**Discussion**

In summary then, these boluses with a density of 3.0 g/cm$^3$ showed improved retention over those in Experiment 5.1B which had a density of 2.5 g/cm$^3$. Their release rate was less than those in Experiment 5.1B and greater than shown in Experiment 5.4. They did however appear to continue to release material until day 76. The quantity of copper supplied daily from the two boluses can be estimated at 32 mg and this gave the bolused ewes larger hepatic copper stores than the control ewes, the majority of which were hypocupraemic.

## Experiment 5.7

## The release rate and retention of a single 19 mm commercial prototype bolus when administered to ewes and lambs.

### Introduction

Increasing the density of the bolus to 3 g/cm$^3$ in earlier experiments had been shown to improve retention. The addition of iron shot to the mould before pressing was however a laborious process and an easier and cheaper alternative way to increase density was required for a prototype commercial product to be developed.

A dispersible end-weight of iron powder with some manganese sulphate added was used in the cattle bolus production from 1989. The development is described in Experiment 3.7. A smaller end-weight which could be used in the 19 mm diameter sheep bolus was also produced. Also for eventual commercial reasons the development of the sheep bolus now focused on administration of a single bolus.

This experiment describes the use of a single weighted bolus in ewes and in lambs. The main purpose of the experiment was to determine retention of the boluses.

### Materials and methods

#### Administration to lambs

One hundred Greyface wether lambs, mean initial liveweight 40 kg were each given one 19 mm bolus administered using an oesophageal balling gun. During administration one bolus was immediately regurgitated otherwise the procedure was carried out with ease.

The boluses were composed of the modified matrix as detailed in Section 2 with an added dispersible end-weight . The bolus dimensions were; weight 42 g, length 4.9 cm, density 2.8 g/cm$^3$. The boluses had a range in weight of 38.8 - 44.1 g. The lambs were not identified as in the other experiments so a comparison could only be made between mean bolus weight at administration and on recovery at slaughter.

After bolusing the lambs were at grass for 7 days before being transferred to fodder rape. After a further 35 days this was exhausted and they were returned to grass.

The lambs were slaughtered in groups at 19, 26, 55 and 90 days after bolus administration. At slaughter the reticulo-rumen of each lamb was searched and the boluses recovered and weighed.

**Administration to ewes**

Twenty-two Greyface ewes, mean initial liveweight 63 kg were each given one 19 mm bolus administered using an oesophageal balling gun. The ewes were identified and the initial bolus weight recorded against each ewe number. The ewes were housed and fed hay and concentrates. One hundred and forty six days after bolus administration the ewes were slaughtered and the reticulo-rumen was searched and the boluses recovered and weighed.

**Results**

**Lambs**

The retention of the bolus in these lambs was 97% which was fully acceptable. The mean weight of boluses recovered at slaughter is given in Table 5.13. Using these mean weights the daily bolus release rates were; days 0-19, 0.31 g; days 20-26, 0.16 g; days 27-55, 0.04 g; and days 56-90, 0.04 g.

**Table 5.13 The mean weight and retention of a single 19 mm diameter bolus when given to lambs and recovered at slaughter, 19, 26, 55 or 90 days after administration.**

| Day | No of boluses | Bolus weight (g) mean | s.d. | Retention % |
|---|---|---|---|---|
| **0** | 100 | 42.1 | 1.07 | - |
| **19** | 30 | 36.2 | 1.77 | 100 |
| **26** | 46 | 35.1 | 1.61 | 100 |
| **55** | 11 | 34.0 | 1.16 | 92 |
| **90** | 10 | 32.7 | 1.19 | 83 |

In Experiment 5.2. the mean total weight loss of a single bolus at day 59 was 4.25 g. In this present experiment at day 55 the figure was 8.1 g. The modified matrix used by the commercial manufacturer was thus performing similarly to that as it did in cattle when it released at around double the rate of Matrix No 1.

It is encouraging that the bolus continued to release material for at least 90 days, and that at 40 mg/day it was releasing 8 mg copper with 0.042 mg cobalt and 0.045 mg selenium. The supply of cobalt and selenium was however well below the target, with less than 20% of the dietary allowance of a 70 kg ewe with twin lambs being provided. The concentration of cobalt and selenium within the bolus matrix could however be increased as was done with the cattle bolus in Experiment 3.10.

**Ewes**

Twenty one of the boluses were recovered giving 95% retention, a similarly high level as found for the lambs. The individual values for initial and final bolus weights are given in Table 5.14.

The mean bolus weight at slaughter was 29.0 g and the mean release rate over days 0-146 was 0.08 g. For the lambs the figure was 0.10 g over days 0-90. Since the release rate is likely to decline over days 90-146 these figures are comparable and certainly do not show any evidence of major difference between lambs and ewes, and between indoor and grass diets.

**Table 5.14 Individual bolus weights at administration and at slaughter for ewes given a single 19 mm diameter bolus.**

| Ewe no | Initial wt day 0 | Recovered wt day 146 | Release rate g/bolus/day |
|---|---|---|---|
| **1** | 41.43 | 31.51 | 0.07 |
| **2** | 39.56 | 28.34 | 0.08 |
| **3** | 40.86 | 25.77 | 0.10 |
| **4** | 40.63 | 29.16 | 0.08 |
| **5** | 41.59 | 32.01 | 0.07 |
| **6** | 44.44 | 30.52 | 0.10 |
| **7** | 40.55 | 29.52 | 0.08 |
| **8** | 40.03 | 29.22 | 0.07 |
| **9** | 40.32 | 30.49 | 0.07 |
| **10** | 40.28 | 26.38 | 0.10 |
| **11** | 40.21 | 29.31 | 0.07 |
| **12** | 40.80 | 26.37 | 0.10 |
| **13** | 41.15 | 31.17 | 0.07 |
| **14** | 41.87 | 28.01 | 0.10 |
| **15** | 40.49 | 32.28 | 0.06 |
| **16** | 40.10 | N.R. | - |
| **17** | 41.13 | 30.93 | 0.07 |
| **18** | 39.25 | 21.13 | 0.12 |
| **19** | 40.50 | 30.11 | 0.07 |
| **20** | 40.73 | 29.18 | 0.08 |
| **21** | 41.43 | 27.91 | 0.10 |
| **22** | 41.37 | 29.36 | 0.08 |
| **Mean** | **40.9** | **29.0** | **0.08** |
| **s.d.** | 1.03 | 2.55 | 0.016 |
| **c.v.** | 2.5% | 8.8% | 19.1% |

N.R. not recovered from the reticulo-rumen at slaughter

# SECTION 6

## Experiment 6.1.
## The release of avoparcin from prototype avoparcin-containing boluses when tested in adult cows.

## Experiment 6.1.

## The release of avoparcin from prototype avoparcin-containing boluses when tested in adult cows.

### Introduction

Avoparcin is a glycopeptide antibiotic whose use as a feed additive for cattle has been reported to increase feed conversion efficiency (Cuthbert, Thickett & Smith, 1984). This improvement is due to the induced changes in rumen fermentation which enhance the production of propionate at the expense of methane and acetate (Ingle, Dalrymple & Kiernan, 1978).

Studies with sheep have also shown a secondary effect with improvements in the absorptive efficiency of protein from the small intestine (Macgregor & Armstrong, 1984).

Avoparcin has been supplied to cattle at grass whether incorporated into supplementary feed or in salt-mineral mixes, with improvements in average daily gain up to 25.6% over unsupplemented control animals. However both these methods of supplementation will result in large variations in avoparcin intake, as has been discussed earlier in this thesis.

An experimental formulation of a rumen bolus was developed by the American Cyanamid Company which would supply a set quantity of avoparcin daily to each individual animal. The formulation of the bolus was barium sulphate, and carnauba wax with 21 g avoparcin in each bolus. The avoparcin was released from the bolus leaving the remaining matrix intact.

It was designed to release 150 mg/day over 140 days. This experiment investigated the release of avoparcin from four different prototype boluses. The release was compared to a control in-feed supplement of 150 mg avoparcin/day.

Virtually 100% of administered avoparcin is not absorbed by the digestive tract and is passed out in the faeces. The release of avoparcin from each of the different bolus formulations was measured by determination of the total faecal output and the concentration of avoparcin in the faeces.

## Materials and methods

### Animals and experimental design

Twenty pregnant Hereford x Friesian beef cows of mean liveweight 474 kg (s.d. 53.7) and 16 weeks before calving were allocated to five equal groups each of four cows on the basis of liveweight and expected date of calving. Treatments of the different bolus types were assigned at random to the five groups. The treatments were:

| Group | Bolus type |
|---|---|
| 1 | I |
| 2 | II |
| 3 | III |
| 4 | IV |
| 5 | In-feed supply of 150 mg/head/day avoparcin |

To act as a standard controlled input of avoparcin (Group 5), Avotan 50 (Cyanamid of Great Britain Ltd) was added to the concentrate given to those cows at a rate of 75 mg/kg FM

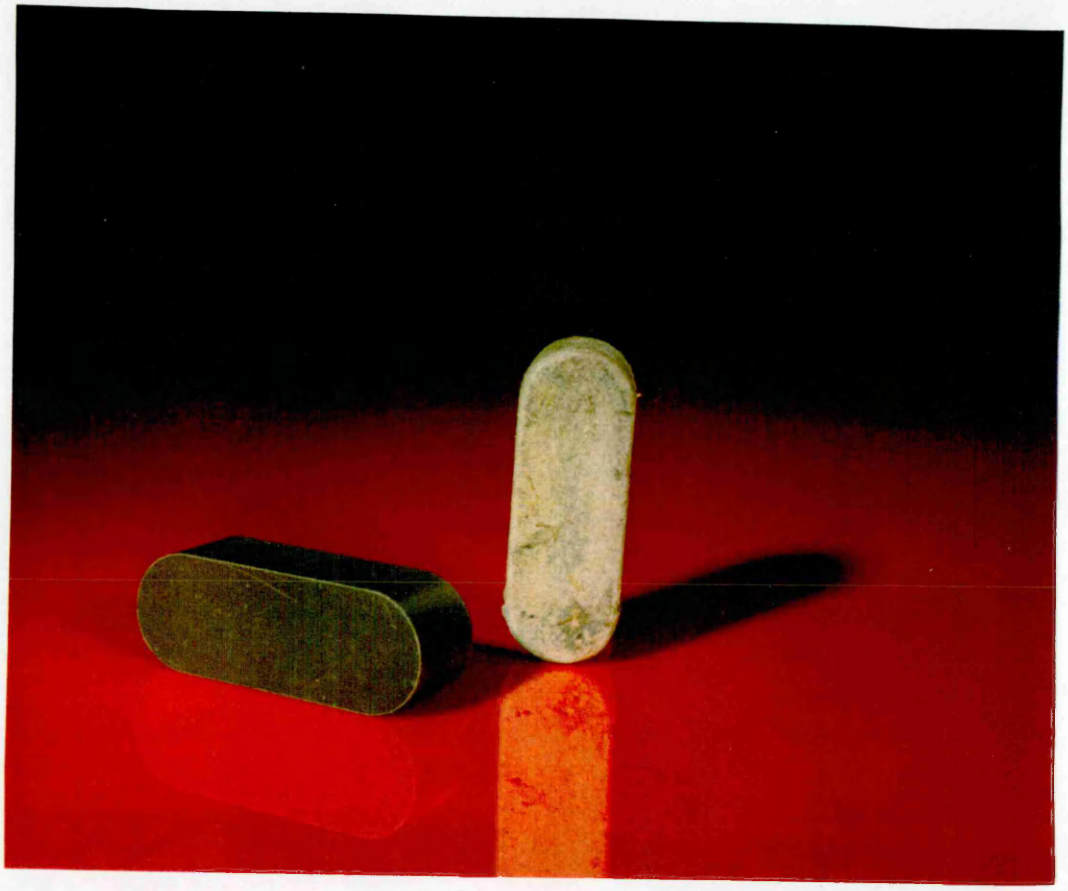
To allow an assessment of faecal dry matter output, chromic oxide was included in the 2 kg concentrate given daily to all the cows to act as an inert faecal marker throughout the whole experimental period. To ensure uniform incorporation, all the concentrate ingredients were ground and cubed.

From the concentration of chromium determined in the total feed DM intake and in samples of faecal DM obtained at regular intervals from each cow assessments were made of faecal DM output to allow for the eventual assessment of avoparcin present in faeces as released from the boluses.

### Boluses and administration

A single bolus of types I-IV supplied by Cyanamid was administered to each of the four cows in each treatment group 1-4 using an adapted oesophageal balling gun. The boluses were similar in appearance but of different composition. They were 3.2 cm wide, 3.2 cm high and 8.2 cm in length and weighed 142 g with a density of 2.0 g/cm$^3$. One such bolus is shown in Plate 16.

**Plate 16.**
**The prototype avoparcin-containing bolus and on the right a bolus regurgitated at grass 8 months after administration.**



## Management and feeding

The cows were housed in a conventional byre in twin standings fitted with facilities for individual feeding. Before calving the cows were given each day 2 kg concentrate and 6 kg hay in two equal feeds (0800 and 1600 h). After calving an additional 1 kg of concentrate was given. After day 141 of the trial the daily concentrate allowance increased to 4 kg.

The mean analyses of the feeds given during the trial are shown in Table 6.1. Concentrate A was given to Groups 1-4, concentrate B to Group 5 and concentrate C (with no chromic oxide or avoparcin) was given to all the cows after calving.

Chromium concentrations in feeds and faeces was determined by the method of Williams, David & Iismaa (1962).

**Table 6.1 Mean analyses (g/kg DM) of the concentrate feeds and hay.**

| | Concentrate | | | Hay |
|---|---|---|---|---|
| | A | B | C | |
| Dry matter (g/kg) | 862.7 | 860.4 | 878.5 | 854.2 |
| Crude protein | 167.5 | 166.5 | 170.4 | 58.6 |
| Crude fibre | 52.5 | 53.7 | 63.9 | 338.2 |
| Ether extract | 8.2 | 8.2 | 15.7 | 8.2 |
| Ash | 68.0 | 65.2 | 38.2 | 49.6 |
| Chromium | 2.94 | 2.96 | 0 | 0 |
| Avoparcin mg/kg | 0 | 63.0 | 0 | 0 |

## Sampling of faeces to obtain chromium concentrations

Chromic oxide was included in the cubed concentrate feed as Curran, Leaver & Weston (1967) considered that a more uniform distribution in faeces was obtained than when given as either a powder added to feed or within a gelatin capsule administered daily with an oesophageal balling gun.

Several previous workers have recorded that combining several grab samples of faeces taken directly from the rectum over a continuous period of seven days gives an accurate assessment of the digestibility of feed dry matter, i.e. with a standard error of less than 3%. For example, Fishwick et al. (1977) combined five samples per day for seven days and Fishwick et al. (1978), Bass et al. (1980), Bass et al. (1981b) and Alawa et al. (1986) found the amalgamation of three samples per day for seven days to be adequately accurate.

McEleney (1985) has shown that a reduced frequency of sampling of faeces could give equally satisfactory results when cows were established on a constant dietary regime. Combining three grab samples of faeces per day for seven days was as satisfactory as combining five samples per day for the same period. Furthermore highly significant correlations were recorded for the chromium concentrations obtained from a single grab sample obtained on one day and the bulked composite of 21 samples obtained by combining three samples per day for seven continuous days.

In the present experiment the sampling procedures adopted were:

(a) For four consecutive days on three separate occasions (days 14-17, 56-59 and 140-143) five grab samples were obtained per day from each cow at 3-hourly intervals from 07.00 to 19.00 hours and thoroughly mixed before analysis.

(b) Additionally, on a single day every 14 days throughout the whole experiment five grab samples of faeces per cow were obtained and mixed before analysis.

During the collection periods the samples were stored in sealed polythene bags in the byre behind each cow.

**Avoparcin assays in feed and faeces**

Each mixed bulked sample of faeces was divided into three subsamples. The first was for the determination of dry matter and chromium. The second (stored fresh at $4^{o}C$) was for the extraction of avoparcin on the following day. The third was stored frozen for any possible repeat analysis.

**Extraction procedure**

1. To 50 g of faeces was added 50 ml of acetone containing 1.5% HCl. Preliminary investigations had shown that this level of HCl reduced the initial pH of the faeces to between pH 1 and pH 2.
2. The samples were then placed on a mechanical shaker and shaken vigorously for 30 minutes.
3. They were then centrifuged for 10 minutes at 3000 rpm and the supernatant drawn off and adjusted to pH 6 by the addition of KOH.

4. 10 mls of the adjusted supernatant was then diluted 1:1 with the buffer $KH_2PO_4$ (pH 4.5) and this diluted extract was then taken for avoparcin assay.

Avoparcin assay was performed by an agar diffusion method (Cyanamid General Method RLA 10950) using Bacillus cereus as the test organism. The general principle is outlined by the Analytical Methods Committee (1979). The assays were conducted in the Department of Veterinary Pharmacology at the University of Glasgow Veterinary School. Feed avoparcin assays were performed by the Microbiology Department of Cyanamid of Great Britain Ltd. The intended concentration of avoparcin in the feed was 75 mg/kg fresh weight.

**Avoparcin output for the in-feed group of cows**

There was some variation in the level of avoparcin in the prepared feed which was reflected in the output in the faeces at the different sampling occasions. Table 6.3 compares the quantity of avoparcin fed with the determined output recorded and Table 6.4 gives the values for the individual cows. The mean output of avoparcin in the faeces was at a similar level throughout the experiment with only isolated exceptions until day 154. The mean amount given (122 mg) and the calculated recovered (124 mg) are in good agreement with low standard deviations. They are however somewhat less than the initial target infeed supplement of 150 mg avoparcin.

**Table 6.3 Quantities of Avoparcin given in the feed and the calculated output in the faeces (mg/day).**

| Day | Avoparcin given | Avoparcin output in faeces | |
|---|---|---|---|
| | | Mean | s.d. |
| **14** | 122 | 115 | 12 |
| **28** | 123 | 106 | 22 |
| **42** | 134 | 117 | 14 |
| **56** | 112 | 91 | 10 |
| **70** | 175 | 162 | 26 |
| **84** | 93 | 106 | 20 |
| **98** | 94 | 109 | 12 |
| **112** | 117 | 127 | 8 |
| **126** | 116 | 151 | 20 |
| **140** | 117 | 155 | 16 |
| **154** | 136 | 123 | 20 |
| **Mean** | **122** | **124** | |
| **s.d.** | 22.3 | 2.9 | |

**Table 6.4. Avoparcin output in the faeces (mg) of the cows given bolus prototypes and those given an in-feed supplement of avoparcin. Type III showed undetectable levels for all cows at all samplings.**

| Days | | 14 | 28 | 42 | 56 | 70 | 84 | 98 | 112 | 126 | 140 | 154 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **In-feed supplement** | | | | | | | | | | | | |
| Cow | 17 | 109 | 121 | 108 | 85 | 143 | 78 | 97 | 135 | 137 | 140 | 105 |
| | 18 | 133 | 74 | 134 | 105 | 164 | 110 | 125 | 131 | 179 | 146 | 107 |
| | 19 | 113 | 121 | 104 | 88 | 198 | 117 | 111 | 124 | 146 | 179 | 143 |
| | 20 | 105 | 109 | 121 | 87 | 142 | 120 | 103 | 117 | 140 | 153 | 137 |
| | mean | **115** | **106** | **117** | **91** | **162** | **106** | **109** | **127** | **151** | **155** | **123** |
| **Bolus Type I** | | | | | | | | | | | | |
| Cow | 1 | 81 | 39 | 58 | 32 | U/D | 42 | * | | | | |
| | 2 | 58 | 40 | 31 | 24 | U/D | U/D | | | | | |
| | 3 | U/D | U/D | U/D | 40 | 63 | U/D | | | | | |
| | 4 | 270 | 175 | 167 | 48 | U/D | U/D | | | | | |
| | mean | **136** | **85** | **85** | **36** | - | - | | | | | |
| **Bolus Type II** | | | | | | | | | | | | |
| Cow | 5 | 130 | 66 | 49 | 1022 | 45 | U/D | | | | | |
| | 6 | 154 | 48 | U/D | U/D | U/D | U/D | | | | | |
| | 7 | 115 | 51 | 54 | 24 | U/D | U/D | | | | | |
| | 8 | 157 | 49 | 47 | U/D | U/D | U/D | | | | | |
| | mean | **139** | **54** | **50** | - | - | - | | | | | |
| **Bolus Type IV** | | | | | | | | | | | | |
| Cow | 13 | 57 | 39 | 75 | 36 | 43 | U/D | | | | | |
| | 14 | 47 | 49 | 81 | 48 | U/D | U/D | | | | | |
| | 15 | 73 | 51 | 75 | 35 | 34 | U/D | | | | | |
| | 16 | U/D | U/D | 23 | 55 | U/D | U/D | | | | | |
| | mean | **59** | **46** | **64** | **44** | - | - | | | | | |

U/D undetectable output of avoparcin.
* After day 84 the faeces of all bolused animals showed undetectable levels of avoparcin.

**Avoparcin bolus groups**

The outputs of avoparcin in the faeces (to day 84) for the individual cows given the different prototype boluses are given in Table 6.4. Bolus Type III is omitted from the table as it showed undetectable levels of avoparcin from all cows at all samplings.

For bolus Type I the daily amounts of avoparcin recovered in the faeces varied greatly from cow to cow. None was detected for Cow 3 for the first 42 days. In contrast the output for Cow 4 reached 270 mg/day at day 14. The mean output for the four cows fell from an initial 136 mg/day at day 14 to 36 mg/day by day 56 after which time the output of avoparcin was generally undetected.

For the cows given Bolus Type II the initial mean daily release of avoparcin was 139 mg but this rapidly declined to about 50 mg. On day 56 a fragment of bolus weighing about 2 g was discovered in the faeces of Cow 5. There was a very high output (1022 mg) of avoparcin from Cow 5 on that day. On days 57 and 58 the outputs were 885 and 222 mg respectively. It was concluded that that particular bolus had disintegrated and about one-third of its total avoparcin content was released in that 3 day period. For all the cows there was generally no detectable level of release of avoparcin after day 56.

The avoparcin contained in Bolus Type IV was released much more slowly over the first 14 days. For Cow 16, avoparcin was not detected in the faeces until day 42. The majority of cows showed no detectable level of avoparcin after day 56.

**Discussion**

Since all the cows were tied in individual stalls and the faeces were carefully examined the apparent failure of all the bolus types to release avoparcin into the faeces after about day 56 (and for the whole period for Bolus Type III) it was assumed not to be due to either regurgitation although the bolus had a very low density or of passage in the faeces.

None of the four types of bolus produced a constant daily release and generally the amounts recovered in the faeces declined markedly after about day 14. Only in occasional samples was avoparcin detected in the faeces after day 56.

By day 56 it was calculated that the mean loss of avoparcin per bolus for Bolus Type I was about 8 g and this assumes that the high output recorded on day

14 occurred from day 0-14. This represents a loss of only about 40% of the total avoparcin present.

At the end of the experiment the cows were transferred to grass. Some 8 months after the initial administration a single bolus was recovered from the pasture. Neither the cow nor the type could be identified. It weighed 136 g and had lost about 6 g from its initial weight. This perhaps supports the general observation that avoparcin release from the boluses generally ceased after about day 56.

It is concluded that none of these bolus types was adequate to provide avoparcin in constant amounts over prolonged periods to cattle. In contrast, in-feed provision of avoparcin appeared to produce constant concentrations in the faeces of the cows.

## GENERAL CONCLUSIONS

The intraruminal bolus system developed in this thesis is suitable for administration to cattle from 150 kg liveweight and with a smaller diameter suitable for administration to sheep from 35 kg liveweight.

The bolus matrix composition and inert polymer coating can be altered to achieve a range of bolus release rates. The release rate and hence the trace elements provided can also be changed by altering the number of boluses administered. Two boluses are recommended as the release of material is partly by dissolution and partly by mutual erosion.

It has been demonstrated that the presence in the reticulo-rumen of metallic weighting devices from other bolus systems may greatly increase the rate of release of material by damage to the polymer coating.

A multiple trace element bolus system developed has an expected life of about 240 days in cattle and this covers a typical grazing season. The amounts of copper, selenium, cobalt, zinc, manganese and iodine released when added to the lowest concentrations of trace elements likely to be encountered in deficient herbage meet the dietary allowances for cattle up to 500 kg as proposed by a Joint Committee of the Ministry of Agriculture Fisheries and Food, Department of Agriculture for Scotland, Department of Agriculture for Northern Ireland, United Kingdom Agricultural Supply Trade Association and the British Veterinary Association (1983).

The copper and selenium supplied by bolus system have been shown to be adequate to prevent and alleviate deficiency of those trace elements as measured by blood parameters. The increase in plasma copper and in blood glutathione peroxidase concentrations were comparable to those associated with alternative supplementation as copper and selenium containing products The problems in determining cobalt status have made it difficult to demonstrate positive responses to the cobalt supplied by the bolus even though with deficient herbage it meets the dietary allowance for cattle up to 500 kg liveweight.

The absence in the reticulo-rumen of any permanent residue and the potentially linear release rate of material from the bolus system would make it a potentially useful vehicle for the delivery of other substances of a medicinal nature. A number of these were examined but only in a preliminary manner. Considerable potential seemed to exist for the inclusion of levamisole hydrochloride, oxfendazole (as anthelmintics) and laidlomycin propionate (as a potential growth promoter) but much further development work would be required.

Very considerable problems were encountered in the development of a comparable bolus system for sheep. These included regurgitation and possible susceptibility to copper poisoning. The presumed economic necessity of use of only a single bolus removing the release of bolus matrix by erosion in addition to dissolution probably implies that a multi-trace element bolus alone or a modified product containing a medicinal product for sheep is not a feasible proposition.

## APPENDIX 1

**Raw material specifications.**

### 1. Copper oxide (CuO)

Source: Twinstar Chemicals Ltd 'sedema cupric oxide'

| | |
|---|---|
| Cu | 77.2% |
| CuO | 96.3% |
| $Cu_2O$ | 1.8% |

Black powder, particle size - 90% less than 44 microns, bulk density 1.78 $g/cm^3$.

### 2. Manganese sulphate ($MnSO_4$ $H_2O$)

Source: Fergusson Wild & Co Ltd 'Feed grade'

| | | |
|---|---|---|
| $MnSO_4$ $H_2O$ | min | 98% |
| Water soluble Mn | min | 31% |
| Fe | max | 0.005% |
| Cl | max | 0.012% |
| Water insoluble | max | 0.05% |
| pH value | | 6-7 |

Pink-white powder 0.03-0.05 mm.

### 3. Zinc sulphate ($ZnSO_4$ $7H_2O$)

Source: Steetley Berk 'Zinc sulphate industrial'

| | | |
|---|---|---|
| $ZnSO_4$ $7H_2O$ | min | 98% |
| Zn | max | 22% |
| Heavy metals | max | 0.05 |
| Cl | max | 1.00% |

White crystalline powder.

**4. Zinc oxide (ZnO)**

Source: K & K Greefe 'EP/M' grade

| | | |
|---|---|---|
| Loss at 110°C | max | 0.10% |
| ZnO | 99.2-99.5% | |
| Pb | max | 0.01% |
| Cu | max | 0.003% |
| Cd | max | 0.0005 |

| | |
|---|---|
| Grey/white powder | |
| Bulk density | 600 g/litre |
| Residue on 325 mesh sieve | 0.01-0.08% |

**5. Zinc oxide (ZnO)**

Source: Vine Chemicals Ltd 'Vidox A.F. grade'

| | |
|---|---|
| Zno | 98% |
| PbO | 0.06% |
| CdO | 0.01% |
| FeO | 0.01% |
| Moisture | 0.15% |
| Acid insol. | 0.40% |
| Water sol. | 1.0% |

| | |
|---|---|
| White powder | |
| Mean particle size | 0.60 microns |
| Retention on 240 mesh sieve | 0.30% |
| Bulk density | 1042 g/litre |

**6. Zinc oxide (ZnO)**

Source: Manchem 'Vitazinc'

| | | |
|---|---|---|
| Zn | min | 72% |
| PbO | max | 0.1% |
| Cu | max | 0.1% |
| Cd | max | 0.001% |
| Fe | max | 0.5% |
| Ca | max | 2-3% |
| As | max | 0.005% |

Brown powder, percent < 250 um - 100%.

**11. Vitamin $A/D_3$**

Source: Roche Products Ltd. Rovimix A/D3 500/100 Type P.

| | |
|---|---|
| Vitamin A content | min 500,000 iu/g (equivalent to 150 mg retinol) |
| Vitamin D3 | min 100,000 iu/g (equivalent to 2.5 mg cholecalciferol) |
| Ethoxyquin content | approx 5% |
| Loss on drying | max 8% |
| Dark brown powder | |
| Particle size | 0.15-0.8 min |
| Bulk density | 0.6 kg/litre |

**12. Dicalcium phosphate dihydrate ($Ca\ HPO_4.2H_2O$)**

Source: Albright & Wilson 'Caliment'

| | |
|---|---|
| Calcium | 31.9-33.5% as CaO on material as it is. |
| Loss on ignition | 24.5-26.5% |
| As | 0.0003% max |
| Pb | 0.0005% max |
| Heavy metals | 0.003% max |
| Cu + Zn | 0.005% max |
| Zn | 0.0025% max |
| F | 0.005% max |

## APPENDIX 2

## Experimental techniques

### 1. Dry matter

The dry matter (DM) of feed liver and faecal samples was determined by heating in a hot air oven at $90^{o}$ C for 24-48 hours until a constant weight was attained.

### 2. Grinding of samples.

All feed, liver and faecal samples were milled after drying. Samples were ground to a size which passed through a 0.5 mm mesh sieve. The dried sample was then mixed thoroughly and placed in an airtight container which was then stored in a darkened room.

### 3. Copper in feed, liver and faeces.

The copper content of feed, liver and faeces 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 required.

### 4. Copper in blood plasma.

This was determined by atomic absorption spectrophotometry (Perkin-Elmer 1976). Plasma was separated by centrifuging the whole blood sample for 15 minutes at 3000 rpm. The plasma was then pipetted off into bijou bottles. 1 ml of plasma was then diluted with an equal volume of distilled water and read on an atomic absorption spectrophotometry at 324.8 nm against standards.

**5. Whole blood glutathione peroxidase.**

The activity of this selenium dependant enzyme was determined using a 'RANSEL' (Randox Laboratories Ltd) kit. The assay was carried out at 37$^{o}$C and the method was based on that of Plagia & Valentine (1967).

**6. Blood plasma Vitamin B12.**

This assay was carried out by the local veterinary investigation centre. The method was by microbiological determination using Lactobacillus leishmanniia.

**7. Chromium in feed and faeces.**

The chromium content of feed and feacal samples was determined by atomic absorption spectrophotometry using the method of William, David & Iismaa (1962).

**8. Statistical Analyses.**

Variations around the mean were expressed as the standard deviation (sd). The standard t test was used to analyse the results with the standard error of the difference (SED) expressed. P values $<0.05$ were considered significant.

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