The factors influencing the availability of cobalt in soils, uptake by herbage and ruminant health and productivity

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By

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TABLE OF CONTENTS

SUMMARY

1. In a series of laboratory experiments involving a total of eighteen soils, the adsorption and desorption characteristics of Co were examined. When the adsorption data from eight soils were applied to various adsorption models, both the Freundlich and Tempkin equations showed good agreement with the observed adsorption pattern. Further, soil pH was identified as the most important factor governing the amount of Co adsorbed and desorbed. For one soil, Co adsorption from a 14.73 μg Co ml^{-1} solution was complete at pH >8.0, whereas, only negligible amounts of this Co was desorbable. For the same soil at pH <5.0, however, all the adsorbed Co was desorbed within a two-hour shaking period with a Co free solution. Both soil texture and organic matter content appeared to have a strong influence on Co adsorption and desorption, with the role of reducible manganese becoming more important in determining the amount of Co desorbed from samples left for periods of between 2 to 4 weeks. However, while an attempt was made to define an adsorption/desorption index for predicting the residual value of Co application to pasture only preliminary suggestions are presented here.

2. In both glasshouse and field investigations the role of pH and grass species in determining herbage Co concentrations was studied. The glasshouse investigation, however, for various reasons failed to show the recognised effects of both pH and species on Co uptake but did suggest that water-logging may have an overriding influence. In addition, this work demonstrated many problems in using pot trials for Co uptake studies. In the field, whereas at one site clover contained the highest Co concentrations of all the species examined this was not the case at another site. This highlighted the problems of using herbage Co values to diagnose Co deficiency with

factors such as soil Co concentration, soil fertility, soil variability, plant type, maturity and part of plant sampled all governing the measured herbage Co content.

3. From field experiments it was found that both the form of fertiliser N and application rate used governed herbage Co concentrations. In general, fertiliser N application reduced herbage Co concentrations, with the most marked effect when nitrogen was applied as nitrochalk rather than as ammonium nitrate or urea. However, when a yearly application of 522 kg N ha^{-1} as ammonium nitrate was applied to Co-treated plots the strong acidifying effect enhanced Co uptake by the plants. Possible reasons behind these findings are discussed.

4. Housed cattle maintained on a Co-deficient diet showed no effect on liveweight performance until some 40 to 60 weeks on experiment despite producing very low serum vitamin B_{12} values after 10 weeks. However, the immune status as measured by the neutrophil function test, was substantially reduced within 10 weeks of commencing the low Co diet. In general, the metabolite methyl malonic acid in serum increased as serum vitamin B_{12} decreased but since the numbers involved in these experiments were small further experimentation is required to determine how useful it is likely to be in the diagnosis of Co deficiency and to define interpretive criteria. Whereas both assay procedures used to measure serum vitamin B_{12} concentrations gave the same trends, the values obtained were on average greater by the radioassay than the microbiological technique. The consequences of this on defining diagnostic limits for serum vitamin B_{12} are discussed.

5. On administration of Ostertagia ostertagi larvae, Co-depleted cattle showed a greater weight loss than Co-supplemented cattle, but showed

no difference in the prepatent period, worm egg production or serum gastrin and pepsinogen concentrations. After anthelmintic treatment both groups showed a similar response.

6. The use of trace element supplemented anthelmintics, although of benefit in supplying Se, proved less effective for Co as the Co additions were too small to maintain increased serum vitamin B_{12} concentrations between the monthly treatment intervals. In contrast, administration of a slow-release Co bolus, monthly 1000 µg vitamin B_{12} injections or monthly 250 mg Co oral drenches, substantially improved serum vitamin B_{12} values above the controls.

7. In a field trial, the application of 0.6 kg Co ha^{-1} as hydrated cobalt sulphate, although failing to enhance soil available Co as measured by extraction with 2.5% acetic acid increased herbage Co concentrations in the first year above untreated pasture. However, in a second grazing season both Co-treated and untreated pasture contained similar Co concentrations. The reasons behind this are discussed in relation to the Co adsorption/desorption findings. In both years, ewes and their twin lambs grazing the Co treated pasture showed enhanced serum vitamin B_{12} values and improved liveweight gains over those animals grazing untreated pasture. The findings, also, showed that grazing Co treated pasture or the administration of a slow-release bolus provided the most cost-effective methods of treating Co deficiency at Upper Auchinlay Farm.

CHAPTER 1 - INTRODUCTION

Although Co deficiency has been recognised as a nutritional disorder in ruminants for around fifty years it still remains a common cause of poor animal productivity in the U.K. The classic symptoms of clinical Co deficiency are well characterised and have been well documented. In the 1950s it was discovered that rather than a direct Co requirement, ruminants require Co in the form of vitamin B_{12} which is synthesised by rumen microbes from Co and various organic precursors. Hence, all the observed symptoms are in fact due to a deficiency of this vitamin. In sheep, these include inappetence, anaemia with associated pallor of the mucous membranes, severe wasting, skin fragility and lachrymation (COSAC/SARI, 1982). In cattle, the symptoms are almost identical but other features such as a rough coat appearance, increased nervousness and depraved appetite are often present (COSAC/SARI, 1982). For both species, severe cases result in a rapid loss of weight followed by death (Underwood, 1977) but such extremes are rarely seen nowadays as affected animals receive Co treatment at an earlier stage in the development of the malady. However, less obvious symptoms such as reduced immune response (COSAC/SARI, 1982) increasing the risk of secondary diseases, such as gastro-intestinal infection, are often not recognised as an effect of a low Co dietary intake.

Co deficiency is found worldwide with incidences reported in Australia, (Gardiner, 1977), New Zealand (Andrews, 1970), U.S.A. (Kubota, 1980), Ireland (Poole et al, 1977) and U.K. (Archer, 1971; COSAC/SARI, 1982). In Scotland, the disease is particularly associated with soils from Old Red Sandstone as found around the Moray and Cromarty Firths and on granites and sandy parent materials near the Solway Firth, with the less severe forms of the deficiency widespread on acid igneous and

arenaceous rocks (Thornton and Webb, 1980). However, on an individual farm basis the incidence of the problem can be sporadic and has resulted in the traditional practice of moving affected animals to so-called "healthy" pastures either on the same or neighbouring farms (Fraser, 1947). Further, various pasture management schemes such as drainage, liming, fertiliser N application and reseeding have been implicated in influencing plant uptake of both native and soil applied Co (Voss and MacPherson, 1977).

Whereas clinical Co deficiency is clearly defined and recognised, the diagnosis of sub-clinical Co deficiency is difficult since no visual symptoms are present (Latteur, 1962). According to COSAC/SARI (1982) such a condition may be of greater economic importance than the clinical form affecting a large but as yet unknown number of animals. Often it goes unnoticed until the introduction of some form of Co supplementation results in improved liveweight gains and general condition of the animals (COSAC/SARI, 1982).

As with all trace element deficiencies it is difficult to determine the number of sheep and cattle affected by either the clinical or sub-clinical form of Co deficiency. However, Mills (1981) using data from the "Veterinary Investigational Diagnosis Analysis Service" (V.I.D.A.S.) found that it ranked between eighth and twelfth in a list of around 130 categorised diseases. In addition, the incidence of the problem is far from decreasing with the number of positive diagnoses made by the V.I.D.A.S. nearly doubling for cattle and increasing by 150% for sheep between 1976-1979 (Mill, 1981) and Mills (1985) proposed that 15-20% of all trace element problems in ruminants in the U.K. may be attributed to a low Co intake.

While there is little data available to determine the extent of the problem in Scotland, COSAC/SARI (1982) suggested that <50% of arable land contains adequate amounts of Co, with the proportion somewhat less in mineral soils of upland and hill farming areas. However, this is likely to change with the increasing use of upland improvement schemes, involving liming, drainage and the introduction of more nutritive grasses, which have been recognised as reducing herbage Co concentrations with a consequent decline in the Co intake of the grazing ruminant (Voss and MacPherson, 1977). Hence, Co deficiency may arise in areas which in the past were regarded as containing sufficient Co.

Although Co deficiency is commonly regarded as occurring on its own, various workers have found enhanced weight responses following Co and Se supplementation (Andrews et al, 1964 ; Wise et al; 1968) or Co and Cu treatment (Voss and MacPherson, 1977) above those obtained when Co, Se or Cu were administered on their own. In contrast, Andrews and Isaacs (1964) failed to find any further improvement in liveweight performance when Cu was administered to Co dosed lambs.

In terms of its identification, a range of diagnostic measures including soil and pasture Co analysis, blood and liver vitamin B_{12} and liver Co determinations are available (Voss and MacPherson, 1977). However, with the increased importance of sub-clinical deficiency there is a need to define new interpretive criteria and produce new measurements which are more sensitive and able to predict sub-clinical Co deficiency. Additionally, although various agronomic practices such as liming, drainage, fertiliser N application and reseeding are known to alter the Co status of pasture, there is a lack of knowledge quantifying their effect. If such information was available it would be possible to determine the economic value of such measures in relation to their effects on Co availability to the grazing ruminant.

While a large number of Co control methods are available, such as regular vitamin B_{12} injections, administration of a slow-release bolus, Co treatment of pasture, little work has been done to compare the merits of the various treatments or the applicability of each to different situations. Further, in recent years, low residual values to Co application to soils (Evans, 1985; Klessa et al, 1988) have been obtained, and this has necessitated the definition of those soil properties which cause this poor response.

In the past, most workers have concentrated on studying either soil/plant (Mitchell, 1964; Klessa et al, 1988) or herbage/ruminant (Whitelaw and Russel, 1979) relationships, rather than the movement of Co from the soil, through the herbage to the grazing ruminant. Therefore, although a substantial amount of work has been carried out on Co there are large gaps in the knowledge as, for example, how animal factors such as soil ingestion, influence the availability of soil applied Co to grazing ruminants.

The aims and objectives of this work are to:

1. Determine the soil properties which have most influence on Co adsorption and desorption and to propose an adsorption/desorption index for use in predicting the likely response to soil applied Co in the field.

2. Quantify the relative effect of soil acidity on the Co concentrations of various grass species and determine the critical pH value above which the Co status of pasture is adversely affected, and to assess the extent to which commonly used pasture species affect the Co content of the sward.

3./

3. Investigate and quantify the effect of fertiliser N application on herbage Co concentrations both in relation to the form of fertiliser N used and the rate of application.

4. Study the relationship between the Co status of cattle and their immune response as measured by the ability of isolated neutrophils to ingest and kill the yeast Candida albicans. Further, to determine whether there is an increased susceptibility of Co-deficient cattle to gastro-intestinal infection with Ostertagia ostertagi.

5. Compare the relative merits of assessing the Co status of cattle by examining the use of serum vitamin B_{12} concentrations (as measured by both microbiological and radioassay) with the more novel approach of measuring serum methyl malonic acid concentrations.

6. Assess the efficacy of treating sheep with Co via a trace element supplemented anthelmintic.

7. Examine, in a field trial, the availability of both native and soil applied Co to the grazing ruminant, i.e. investigate the movement of Co from the soil to the ruminant via the herbage. In addition, to compare the relative merits of soil, herbage and blood measurements for diagnosing the Co status of the grazing ruminant.

8. Compare the cost-effectiveness of different methods of Co supplementation, namely, monthly vitamin B_{12} injections, administration of a slow-release bolus at the start of the grazing season and Co application to pasture.

CHAPTER 2 - LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND TO COBALT DEFICIENCY IN RUMINANTS (up to 1951)

For hundreds of years, farmers across the world have found some pastures to be unsuitable for grazing sheep and cattle. Even on apparently rich and abundant pastures, ruminants lost their appetite, became weak and emaciated before eventually dying, but it was not until 1951 that the cause was attributed to a lack of vitamin B_{12}, the biologically active form of Co.

Early this century pioneering studies into the cause of this ill-thrift were carried out in New Zealand and Australia and have been reviewed by Hopkirk and Patterson (1954) and Fraser (1947).

Since the observed ill-thrift could be cured or prevented by moving the diseased animals to so-called "healthy pastures" it was realised that the cause of the disease lay in an undefined deficiency, rather than in an infection.

As later stages of cobalt deficiency are characterised by severe anaemia, New Zealand researchers suspected iron (Fe) deficiency as the cause of the wasting disease "Bush-sickness". Various iron salts were tested and found to be an effective means of treating affected animals, with limonite (bog-iron) being a particularly inexpensive and successful form.

However, workers in Australia found that limonite was not always effective. Fractionation studies found iron-free limonite cured "Denmark Diseases" or "Enzootic Marasmus" which had similar symptoms to "Bush-sickness". Continued fractionation isolated

four possible curative elements, namely nickel, cobalt, manganese and zinc. Further experimentation showed that supplementation with Co cured "marasmus", and "coast disease".

Once Co deficiency was isolated as the cause of "wasting" diseases in Australia, a number of other diseases were soon recognised as being linked to cobalt deficiency. These diseases were often named after the district where it occurred or from the symptoms seen (Table 2.1).

By 1951 the active form of Co in ruminant metabolism was identified. Two independent groups of workers in England and the United States, found that anti-pernicious anaemia factor, subsequently named vitamin B_{12} and isolated from the liver, contained 4% Co (Smith, 1948; Rickes et al., 1948). Three years later Smith et al (1951) showed conclusively that pine in lambs could be cured by subcutaneous vitamin B_{12} injections. These findings were confirmed by Hoekstra et al (1952) and from further studies it became clear that Co deficiency in ruminants was actually a vitamin B_{12} deficiency.

Dietary Co is utilised by the rumen microbes in the synthesis of vitamin B_{12} which is then absorbed and used in various metabolic processes. When Co intake is restricted, microbial vitamin B_{12} production declines with a subsequent reduction in vitamin B_{12} availability to the animal.

2.2 SOIL FACTORS INFLUENCING COBALT STATUS

The Co contents of soils are expressed either as total or available concentrations. Total Co is defined as the weight of Co per unit weight or volume of soil obtained after treatment

Table 2.1 Local names for "ill-thrift" diseases caused by cobalt deficiency [based on Fraser (1947)]

Country	Local name for Co deficiency
New Zealand	Bush-sickness "Morton mains disease" Mairoa dopiness
Australia	Coast disease Wasting disease Enzootic marasmus Denmark disease
Kenya	Nakuruitis
U.S.A.	Salt lick Grand Transverse Neck-ail
Ireland	Galar Truagha
Scotland	Pining Vinquish Daising
England	Moor sickness Pining

with either concentrated acids or fusion with salts (Young, 1979). Such treatments include aqua-regia, perchloric and nitric acids. Available Co is defined as the weight of Co per unit weight or volume of soil available for plant uptake and is estimated by extraction either with dilute acids or salt solutions. In most soils only around 3-20% of total Co is available for plant uptake (Young, 1979). The total Co contents of most British soils lie in the range 1-100 mg Co kg^{-1}, with those <10 mg Co kg^{-1} likely to produce Co deficient herbage, i.e. herbage Co concentrations will be insufficient to meet the metabolic requirements of the grazing ruminant (Thornton and Webb, 1980)

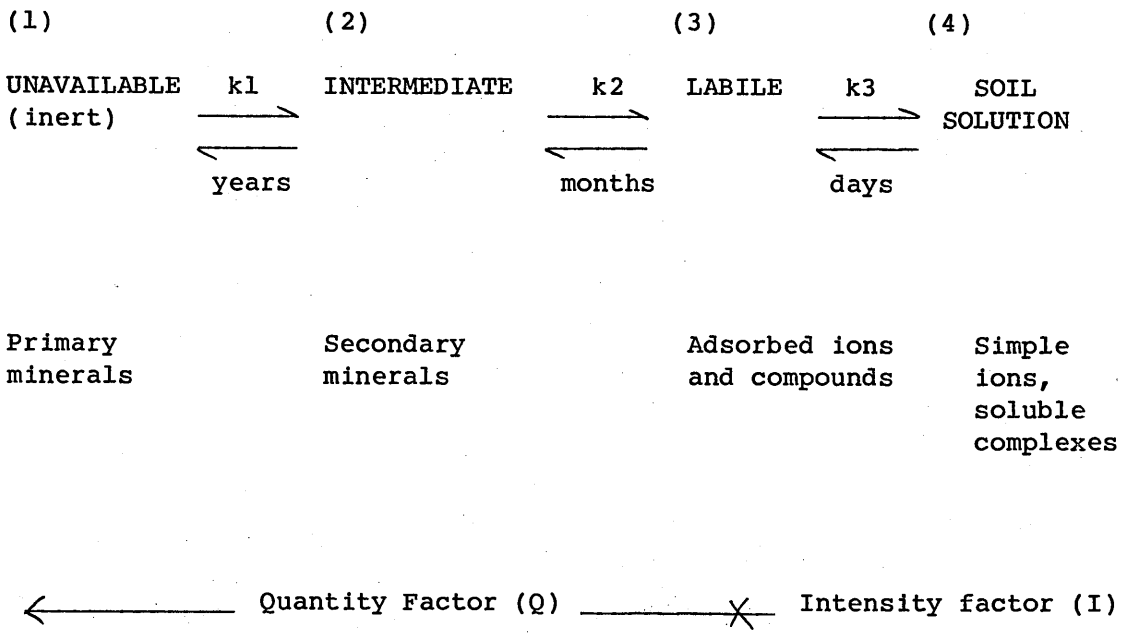
2.2.1 Distribution of Co in the lithosphere

Within the soil, Co can exist in non-labile (i.e. unavailable to plants) or labile (i.e. available to plants) forms. Little information is available on the amount or forms of Co present in each of these groups. West (1981) has summarised how the different trace element pools interact (Fig. 2.1).

Co "locked" within the primary minerals and many of the secondary minerals will be made available to plants over a long time-scale by weathering. Co found in association with sesquioxides, immobilised within complexes, or bound within the lattices of secondary minerals, is unavailable to plants during the growing season. Such forms are more labile than Co found within primary minerals and can release Co to the labile pool over a period of months.

Exchangeable Co, held on negatively charged sites of sesquioxides, clay or organic matter, is readily available to plants. As solution Co concentrations decline (i.e. the intensity factor falls), exchangeable Co may be released to replenish the soil solution.

Figure 2.1 The relationship between the different forms of soil Co [West (1981)]



According to Figure 2.1, Co forms depicted as 1, 2 and 3 dictate the potential supply of Co to plants and are termed the quantity factor. Form 4 represents the immediate source of Co to the plant roots and is termed the intensity factor. Over a growing season, the quantity factor will buffer the soil solution. In practice most of this will be derived from labile exchangeable Co with a small contribution from the weathering of primary and secondary minerals.

Co present in the soil solution is immediately available for plant uptake. The amounts present are very small of the order of 4.12×10^{-4} - 0.01 mg Co l^{-1} with around 25% present as soluble organic complexes (Jenkins and Jones, 1980). The concentration of Co in soil solution is determined by both the pH and Eh of the soil (see section 2.2.2).

(a) Parent Material (Geological Aspects)

Soil Co originates from parent material and on weathering releases Co into the soil solution. The concentration of soil Co derived from different parent materials has been discussed by West (1981).

Igneous rocks make up nearly 95% of the earth's crust (Mitchell, 1964) and form from the solidification of molten silicate magma leading to rocks of highly variable mineralogy. Amongst the forms which are recognised are the ferromagnesian-rich ultra-basic and basic rocks (e.g. serpentine), intermediate rocks (e.g. andesite) and silica-rich acidic rocks (e.g. granite). During formation, various trace elements become incorporated into mineral lattices, the concentration and elemental form being governed by a range of factors, one of the most important being the ionic radii of the elements. The major cationic species present in ferromagnesian minerals are Mg^{2+} (ionic radius = 7.8 nm) and Fe^{2+} (ionic radius 8.3 nm). Ions, such as Co^{2+}, with an ionic radius of 8.2 nm can

substitute for Mg^{2+} and Fe^{2+} and become incorporated into these ferromagnesian lattices (Mitchell, 1964). Hence, ultrabasic and basic rocks, being rich in ferromagnesian minerals, are also relatively rich sources of Co, whereas acidic rocks, like granite, which do not contain ferromagnesian minerals, contain little Co.

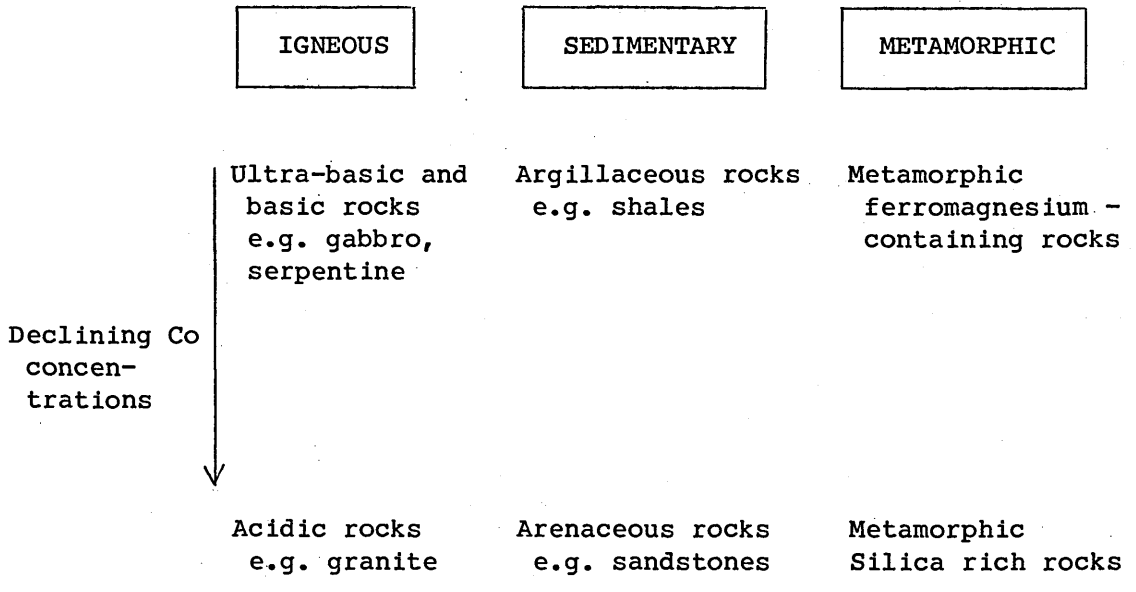
Sedimentary rocks form by hydrological, glacial, aeolian and chemical weathering of previously weathered and unweathered rocks and their constituent minerals. Of the igneous rocks, the most easily weathered are the ultrabasic and basic types which on sedimentation produce Co rich argillaceous rocks, e.g. shales, while the more resistant acidic rocks form silica rich arenaceous sediments, e.g. sandstones.

Metamorphic rocks form when extreme pressure or high temperatures are applied to rocks. The influence of metamorphism on trace element contents has received little attention (Jenkins and Jones, 1980). As the process is generally isochemical, total Co concentrations will remain unchanged, but such strong physical changes may increase the resistance of the rock to weathering and so decrease the supply of Co during soil development. However, when granitisation occurs, Co depletion has been observed (Jenkins and Jones, 1980).

The relationship between rock type and its Co-status is summarised in Figure 2.2. Hence from a knowledge of local geology, it is possible to explain the distribution of inherently Co deficient soils in Scotland (Mitchell, 1960 ; Thornton and Webb, 1980; COSAC/SARI, 1982):

(i)/

Figure 2.2 Generalized diagram illustrating the relationship between rock type and total Co



(i) *Soils formed from arenaceous rock, old red sandstone:*

(a) Large areas in North-east (in particular Black Isle and Caithness areas).

(b) Some inland areas of Central Scotland (particularly in parts of Stirling, Lanark and Perth).

(c) Arable areas found in the South-east (particularly the Tweed Basin).

(ii) *Granitic soils:*

(a) Areas around the Solway (Criffel granite).

(b) Certain Aberdeenshire granites.

(c) Granitic areas on Arran.

(iii) *Various arenaceous deposits:*

Large areas across the Southern Uplands.

(iv) *Metamorphic granites and arenaceous rocks (quartzite):*

Coastal areas of the North-west.

(b) Mineralogy

Primary minerals are defined as unaltered or unweathered minerals which form by magmatic, hydrothermal or metamorphic processes. Two important groups of primary silicate minerals can be recognised with respect to their Co contents:

(i) ferromagnesian minerals, and

(ii) calcium, potassium and sodium aluminosilicates.

Ferromagnesian minerals contain rich deposits of Co from isomorphous substitution of Fe^{2+} and Mg^{2+} within the crystal lattice. In the case of aluminosilicates, i.e. felspar, pyroxenes and amphiboles, from simple ionic considerations it can be seen that Co^{2+} (ionic radius = 8.2 nm) cannot replace Ca^{2+} (ionic radius = 10.6 nm),

K^+ (ionic radius = 13.3 nm), Na^+ (ionic radius = 9.8 nm) or Al^{3+} (ionic radius = 5.7 nm) (Mitchell, 1964), although account must also be made of charge, bonding energies and arrangements in any rigorous explanation. It is not surprising to find, therefore, that aluminosilicates tend to contain relatively low Co concentrations (Mitchell, 1964).

From a knowledge of the major constituents of primary minerals it is possible to predict the likely abundance of Co. This is illustrated in Table 2.2, using data on the major constituents of common primary minerals found in igneous rocks (Mitchell, 1964).

Secondary minerals of which the clay minerals form an important group in soils, are defined as those minerals which form from the weathering of primary minerals. Little information appears to have been published on Co concentrations within different clay minerals.

Clay minerals are composed of sheets of oxygen or hydroxyl ions sandwiched together to produce a layer-lattice structure. Two types of oxygen layers have been recognised which when placed together produce either tetrahedral or octahedral holes, which are occupied by various cations either in 4 or 6 co-ordination with oxygen, respectively. Only small atoms of radii around 3 nm can enter the tetrahedral holes and these are restricted to mainly silicon and some aluminium ions. However, atoms with an ionic radius of around 6 nm can be found within the octahedral bonding sites. These are normally occupied by Al^{3+} (ionic radius = 5.1 nm), Fe^{3+} (ionic radius 6.4 nm), Mg^{2+} (ionic radius = 6.6 nm) (Russell, 1973). It is possible that trivalent Co with an ionic radius of 6.3 nm could replace these ions by isomorphous substitution,

Table 2.2 Elemental composition of primary silicates

Mineral	Major cationic constituents	Presence of Co
Olivine	Mg, Fe	Present
Horneblende	Mg, Fe, Ca, Al	Present
Augite	Ca, Mg, Al	Present
Biotite	K, Mg, Fe, Al	Present
Apatite	Ca, P, F	Absent
Anorthite	Ca, Al	Absent
Andesine	Ca, Na, Al	Absent
Oligoclase	Na, Ca, Al	Absent
Orthoclase	K, Al	Absent
Muscovite	K, Al	Absent

but this form in solution is unstable being reduced to Co^{2+} (Cotton and Wilkinson, 1976) and will not occur in the conditions found during clay synthesis. Co^{2+}, on the other hand, with an ionic radius of 7.3 nm may be involved in some substitution reactions (Russell, 1973). Co incorporation into clay minerals is likely to be restricted to Fe and Mg-rich clay minerals such as the 2:1 clay minerals, i.e. hydrous micas (e.g. vermiculite, illite) and smectites (e.g. montmorillonite), but not the 1:1 clay minerals, i.e. kaolinite. West (1981) suggested that rather than incorporation into the clay mineral lattice, Co is held by the cation exchange surfaces of the clay minerals. Although such forms are strongly held they are more available than occluded forms.

(c) Soil

Various attempts have been made to determine the affinity of Co for the different adsorption sites present in the soil by various adsorption/desorption studies in which known amounts of solution Co are incubated with either the whole soil (Borggard, 1987) or soil components (McLaren, 1986). From soil studies, the clay fraction and iron oxides appear to be the most important adsorption sites (Borggaard, 1987), but McLaren (1986) found clay minerals adsorbed only small amounts compared with hydrous oxides and organic matter. Since Co held by organic matter is readily desorbed (McLaren, 1986) as solution Co concentration declines, Co will be released and some Co will become re-adsorbed by the hydrous oxides, where it is more strongly bound (McLaren, 1986).

A./

A. Mineral Fraction

(i) *Soil texture*

Many workers have shown a strong association between total soil Co and the silt and clay sized fractions (Nahhab and Bhatti, 1958; Reddy and Mehta, 1962; Randawa et al, 1964; Singh and Singh, 1966; Mistra and Kishore, 1967; Rana and Quellette, 1967; Yadav et al, 1975, 1978). This ties in with the fact that in sandy soils there is a higher incidence of Co deficiency (Kubota, 1968). Berrow and Ure (1985) found that although 10-55% of the total Co present in a soil was associated with clays, a substantial amount remained within the primary minerals of the sand fraction.

As well as direct incorporation of Co into clay minerals during synthesis, clay particles play an important role in cation exchange reactions. Various investigations have been made to determine cation adsorption/exchange mechanisms. Hodgson (1960) proposed three types of exchange sites:

1) Exchange sites where most cations are readily adsorbed/desorbed.

2) Specific exchange sites where only certain cations such as Cu^{2+} and Zn^{2+} are adsorbed, but which can exchange with Co^{2+}.

3) Specific sites on which only Co becomes adsorbed to become unavailable.

Early work suggested Co was adsorbed as the mono-valent hydroxyl-cobaltous ion ($CoOH^{+}$) (Spencers and Greseking, 1954) and the following reaction was proposed:

$$\text{Exchanger} - Ca^{2+} + 2\ Co^{2+} + 2\ H_2O \longrightarrow \text{Exchanger} - (CoOH^{+})_2 + Ca^{2+} + 2\ H^{+}.$$

However, Hodgson et al (1964) doubted such a mechanism. Their studies showed that no charge was present at the Co bonding sites and hydrolysis did not occur during the exchange process.

Various workers have established Co reactivity tables for different minerals in the pure form (Kubota and Beeson, 1961; Tiller et al, 1963; Kubota and Pendias, 1973) and proposed the following order for ease of exchangeability of adsorbed Co.

muscovite > haematite > bentonite or kaolinite

In addition, McLaren et al (1986) found that montmorillonite could adsorb almost a hundred times more Co than kaolinite or illite. However, little difference has been shown by other workers in the Co exchange capacity of soils containing different clay minerals (Tiller et al, 1963). This has been partly explained in terms of the iron coatings present on the mineral surfaces masking the exchange sites present on the clay mineral surface (Hodgson et al, 1969). When such coatings are removed exchange/adsorption reactivity for Co increases (Hodgson et al, 1969), suggesting that although clays can hold Co, most of the Co is associated with the oxide precipitates on the clay surface.

(ii) *Hydrous oxides*

Over the last twenty years, the role of manganese oxides on the Co content of soils has received much interest. Australian workers studying intensely weathered soils were

amongst the first to note the strong relationship between Co and manganese oxides (Taylor and McKenzie, 1966). Taylor (1968), using soils from around the world further demonstrated that Co was found in association with the manganese minerals, birnessite and uthiophorite. This stimulated various studies into the mechanism and strength by which Co was held and McKenzie (1970) proposed a reaction mechanism based on crystal field theory. Although this was originally formulated as the mechanism by which Co added in salt solutions was fixed by manganese minerals, a similar mechanism could occur during manganese oxide synthesis. This process appears to have two stages viz:

1) Co^{2+} is rapidly adsorbed onto the surface of oxides by simple cation exchange reactions, displacing Mn^{2+}, K^{+} and H^{+} ions.

2) Co^{2+} migrates into the mineral lattice where a redox reaction takes place:

$$Co^{2+} \longrightarrow Co^{3+} + e^{-}$$

$$Mn^{3+} + e^{-} \longrightarrow Mn^{2+}$$

in which Co enters the mineral structure displacing Mn. The Mn^{2+} ion produced moves to the surface and can take part in further exchange reactions. However, such a mechanism has been criticised as being over-simplified, in that the reaction is considered as a simple substitution of two similarly charged species (Burns, 1976). In addition, no account is made of the relative abundance of Mn^{3+} and although the trivalent form will exist within the lattice, Mn^{4+} will be present in much greater concentrations. Burns (1976) also pointed out the need to consider the size of the substituting ions. He proposed that the large discrepancy in ionic radii between low-spin Co^{3+} and high-spin Mn^{3+} would make displacement difficult and he suggested that substitution occurred between the similarly sized

Co^{3+} and Mn^{4+}. Irrespective of the exact mechanism, manganese oxides appear to have a strong affinity for Co (Murray et al, 1968; Loganathan and Burau, 1973; Murray, 1975; Murray and Dillard, 1979; Traina and Doner, 1985).

In the U.K., where soils are at a much earlier stage of development, the types of manganese minerals present differ markedly from those found in Australia. Nevertheless, Jarvis (1984) concluded that significant proportions of soil Co in England were associated with manganese oxide minerals and he further suggested that such a relationship may explain the sensitivity of Co to changes in acidity and redox potential. This was verified by McLaren et al (1986), from fractionation studies, who showed that soil Co is associated mainly with the soil oxide fractions and in particular manganese oxides.

Early workers concentrated on the relationships between Co and iron oxides rather than manganese oxides. Fujimota and Sherman (1950) observed the highest Co concentrations in the A horizons of soil profiles which were positively correlated with the amounts of iron oxides present. Others have observed an association between total Co and total Fe concentrations in Indian soils (Reddy and Mehta, 1961, 1962; Singh and Singh, 1966; Mistra et al, 1967). In a recent study, Borggaard (1987) found that, although clay presents the most important site for Co adsorption, the remainder of adsorbed Co can be attributed to iron oxides. The iron oxide component can be considered to be composed of two fractions ; an EDTA-extractable fraction showing high reactivity to Co and a residual less-reactive fraction extracted by dithionite (Borggaard, 1987).

B. Organic Matter

The relationship between Co and organic matter has been investigated by different workers. Soil Co contents, either measured as total or available Co, have been found to be correlated with soil organic matter or organic carbon (Bahhe and Zonde, 1962; Rana and Quellette, 1967; Singh and Singh, 1973; Yadav et al, 1975; Elarashidi et al, 1979). However, others (Wright and Lawton, 1954; Reddy et al, 1962; Yadav et al, 1978) have found no relationship between soil Co and organic matter contents.

In general, peat soils are low in Co and deficiency diseases of livestock are common on such soils (Young, 1979). Finch (1978), however, when studying the Co content of peat bogs found that out of a total of ten sampled, only half were deficient in Co, with the rest regarded as containing adequate Co. Thus, broad generalisations cannot be made and consideration must be given to pedogenesis. For example, basin peats are soligenous and if the surrounding area is formed from base rich material, are likely to contain relatively high concentrations of Co. On the other hand, raised bogs and hill peats receiving water solely from rain will be low in Co.

The total Co content of soil organic matter will be influenced by the type of vegetation from which it originates. According to Young (1979) humic material of woody origin contains inherently higher Co contents than that formed from decomposed grass. As humic material is of a highly complex and variable nature, significant quantities of Co may be immobilised in organic structures which are resistant to both biological and chemical breakdown. Such materials have received little, if any, attention in relation to Co availability.

Organic matter can influence Co availability in two ways:

1) Chemisorption of exchangeable Co to negatively charged sites of humic material.

2) Formation of soluble organo-metal complexes.

Humic and fulvic acids appear to contribute most to the ability of the organic fraction to bind metallic ions (Mitchell, 1964). Humic acid forms insoluble complexes, while fulvic acid, which is found in greater proportions down the profile, has a higher complexing capacity than humic acid and the compounds which form are normally water-soluble (Wild, 1988).

Various workers have investigated the role of Co organo-complexing in influencing plant availability when soils are of differing drainage status (Mitchell and Burridge, 1979; Berrow and Mitchell, 1980; West, 1981). This has been carried out by comparing the amounts of Co which are extracted by acetic acid or ethylene diamine tetra acetic acid (EDTA) in freely-drained and gleyed soils (Berrow and Mitchell, 1980). Acetic acid extractable Co will give an indication of inorganically-bound Co, while EDTA, a powerful chelating agent, the amount of organically-bound Co. In a freely drained soil, most of the extractable Co is found in the A horizons, with acetic acid being the most effective extractant, suggesting a considerable proportion of the Co is associated with the inorganic rather than organic component (West, 1981). West (1981) further showed that, although the organic rich surface horizons of a gley soil contained more chelated or complexed Co than the freely-drained soil, substantial amounts were still inorganically bound. Hence even although soil organic matter does contain potential Co exchange sites, Co appears to have a greater affinity for inorganic binding sites.

Little published data is available on the forms or relative importance of soluble organo-Co complexes. Using pure Co salt solutions, Hodgson et al (1965) found little complexation occurred when chelating agents such as EDTA were added. However, Mitchell (1964) identified some Co-fulvic acid chelates from peats and the formation of inert complexes between Co and the fulvic material was demonstrated by MacCarthy and O'Cinneide (1974). They suggested the possible formation of highly stable cobaltic (Co^{3+}) organic chelates, under anaerobic conditions. Schnitzer and Hansen (1970) determined the stability constant (K) of Co-fulvic acid complexes at different pH levels and at different ionic strengths. From their work they produced an order of stabilities at low pH of:

$Fe^{3+} > Al^{3+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Pb^{2+}$

$= Ca^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+}$

which suggested Co could form fairly stable fulvic complexes, with stability increasing with a rise in pH and low ionic strength.

(d) Co contents found in different soil types

During soil development various changes take place which alter the distribution of Co within the soil profile. Various workers (Reddy and Mehta, 1962; Badhe and Zende, 1962) noted the accumulation of Co in the lower horizons of podzols. Walsh et al (1956) explained such observations as leaching of Co from upper horizons, translocation down the profile in association with iron or aluminium or as an organic complex. Berrow and Ure (1985) did not find an enrichment of Co in the thin iron pan of a peaty podzol, but did find an accumulation of Co in the lower horizons. This suggested a greater mobility of Co within the profile than iron.

The combination of podzol development on coarsely textured soils (which are inherently low in Co) and the leaching of substantial amounts of Co beyond the rooting depth, increases the incidence of Co deficiency in podzolic areas (Walsh et al, 1956; Nicolls and Honeysett, 1964; Kubota, 1965). Brown earths forming on clay-rich soil, of naturally higher Co contents, and reduced leaching, contain higher Co concentrations in the upper horizons (Fujimoto, 1950).

2.2.2 Factors influencing soil Co availability

(a) Soil pH

It has been known for some time that soil acidity is of major importance in determining soil Co availability. According to West (1981) soil pH is regarded after drainage as the most important soil factor determining the availability of Co. Many workers have obtained decreased herbage Co concentrations after liming (Ekman, 1952; Hill et al, 1953; Wright and Lawton, 1954; Percival et al, 1955; Archer, 1955). An increase of one pH unit (from pH 5.4 to 6.4) almost halves the herbage Co concentration of a mixed pasture (Mitchell, 1972), but increasing the pH above pH 6.0 has been found to have little further effect (Wright and Lawton, 1954; McLaren et al, 1987; Klessa et al, 1988). This ties in with the early recommendation of Mitchell (1954), who suggested that as long as liming did not increase soil pH above 6.0, most Scottish soils should produce herbage of adequate Co content for the grazing ruminant.

A greater incidence of Co deficiency in grazing animals has been found in naturally occurring high pH soils. For

example, Finch and Rogers (1978) found Co deficiency to be more likely to occur in soils formed on limestone, while Mitchell (1954) explained the prevalence of Co deficiency in the Western Isles by the abundant supply of free $CaCO_3$ released from sea shells.

Recent workers have attempted to define the relationship between soil pH and herbage Co concentrations by various relationships. Klessa et al (1988) found an inverse logarithmic relationship with the gradient dependent on both season and fertiliser N rate. Such a relationship was also obtained by McLaren et al (1987) but was improved further when a curvilinear model was used to take account of the reduced Co herbage concentrations of pH values above 6.0.

The influence of pH on herbage Co concentrations is due to a combination of factors. Liming will not only reduce Co availability by causing greater adsorption onto the various exchange sites and resulting in the precipitation of various Co salts, but increased pH will encourage the establishment of better quality grasses having a lower Co content (Mitchell, 1954).

Soil pH influences the solubility of various Co salts. Young (1949) using pure salt solutions, found all Co salts were very soluble under acidic conditions, with precipitation as hydrated cobaltous salts occurring in the presence of most common anions (e.g. Cl^-, NO_3^-, PO_4^{2-}) in salt solution in the pH range 6.5 to 7.5, with complete precipitation at pH $\geq$8.5. Presumably, since all nitrate salts are soluble, the precipitate which forms in the presence of nitrate is cobalt hydroxide.

The availability of soil Co declines as pH increases. Sanders (1983) investigated both the concentration and forms of Co present in soil solution as pH was adjusted between 5.0 and 7.5. His

results indicated that as pH increased, the concentration of Co in the soil solution declined, with the proportion present as the free divalent ion falling disproportionately. Whereas, divalent Co^{2+} is the major form present at pH 5.0, at higher pH levels there is increasingly more Co present as soluble-organo complexes (Sanders, 1983). In addition, the concentration of isotopically exchangeable Co remained unchanged regardless of soil pH, but Co uptake by Sudan grass declined as pH increased (Gillo and Graham, 1971), suggesting that the Co form at higher pH is less readily taken up by plant roots.

With a rise in pH, there is an increase in the cation exchange capacity of clay, sesquioxide and organic matter. Co adsorption occurs on negatively charged hydroxyl groups found in clays and hydrous oxides and the dissociation constant (pK) for such hydroxyl groups is around 6.0 (Wild, 1988). Hence, for soils of pH >6.0 the negative charge will increase leading to an increase in cation exchange capacity. Maes and Cremers (1975) illustrated this concept by studying the cation exchange capacity of montmorillonite in relation to Co adsorption at different pH levels. They found a low and reversible exchange of Co up to pH 6. By adding the same quantity of Co further increases in pH resulted in 100% adsorption with a significant proportion of the Co held irreversibly. However, when pH was reduced, desorption was rapid even for the Co which had been held irreversibly.

As reviewed earlier (see section 2.1.3 (a) (i)), the strong association of Co with manganese oxide will have a major effect on Co availability. This was investigated by Loganathan et al (1977) using a synthetic hydrous-manganese oxide. At pH <5, structural manganese was released into solution and Co adsorption occurred

specifically at the surface by exchange with bound hydrogen. At pH >6 adsorption increased dramatically. Two possible reasons have been suggested (Loganathan et al, 1977):

(i) The hydroxylate cation ($Co(H_2O)_5.(OH)^+$) found in greater amounts at high pH may show more affinity for the manganese surface than the hydrated species ($Co(H_2O)_6^{2+}$).

(ii) Precipitation of Co hydroxides may occur at the surface at high pH.

Irrespective of the exact mechanism, Loghanathan's work illustrates how the amount of Co adsorption on to manganese oxides is pH dependent. In soil, the situation is more complex, involving a combination of adsorption sites, including clay, sesquioxides and organic matter and different aqueous forms of Co (including soluble organo-metal complexes).

(b) Pedological drainage status

Soil drainage status has a major influence on the amount of Co which is plant available. Early workers were quick to note the higher herbage Co concentrations of poorly-drained as opposed to well-drained soils (Hill et al, 1953). Detailed studies by Mitchell et al (1957) illustrate the strong influence of drainage class on Co availability. Over a two-year study, soil and herbage were taken from both a freely and poorly-drained soil developed on argillaceous schist (Table 2.3). Mitchell et al (1957) proposed that the results in Table 2.3 could be explained by the increased rate of mineral weathering in poorly-drained soils (particularly of ferromagnesian minerals) and the increased presence of soluble organo complexes when soils are waterlogged. Similar increases in herbage Co concentrations under poor drainage conditions were seen by Walsh

Table 2.3 Relationship between drainage status and soil Co availability and plant uptake [extracted from Mitchell *et al* (1957)]

Drainage class	Available Co (measured by acetic acid) mg kg^{-1}	Co content of mixed herbage mg kg^{-1}
Freely-drained	1.0	0.12
Poorly drained	2.7	0.86

et al (1956), and Berrow et al (1982). This was further demonstrated by the fact that when herbage was grown in water-logged pots very high herbage Co concentrations were obtained (Adams and Honeysett, 1964), with plant availability as measured by soil extractants, remaining elevated even after air-drying.

Different extractants have been used to determine the Co forms found in the horizons of both freely and poorly-drained soils. Work by Berrow & Mitchell (1980) indicated that in freely-drained soils, the highest concentrations of available Co are found in the surface horizons, associated with decomposing secondary minerals, sesquioxide or manganese oxides. In poorly-drained soils, surface horizons contained some chelated Co but the highest Co concentration was found in gleyed horizons, firmly bound by "mineral exchange-active material". For all the horizons examined, poorly-drained as opposed to freely-drained soils contained significantly more acetic acid, ammonium acetate and EDTA-extractable Co.

Various suggestions can be made as to how impeded drainage increases Co availability (Mitchell et al, 1957):

(i) Under anaerobic conditions, decomposition of ferro-magnesian minerals and sesquioxides is faster, leading to a greater release of Co into the labile pool.

(ii) Organic matter breakdown is slowed down producing different degradation products which may complex Co in plant available forms.

2.2.3 Assessment of Co availability

The uptake of Co by plants provides the definitive measure of plant availability. However, plant species differ in their rate of Co uptake (see section 2.3.1) and the same single species growing under varying soil conditions (e.g. pH, drainage status, fertility) will differ in its Co uptake. In addition, seasonal changes and variations in Co distribution within the plant, make an assessment of Co availability in terms of plant composition difficult. Whereas, soil sampling can be done at any time during the year, herbage samples can only be obtained during the growing season. Thus, soil Co availability is measured by the use of chemical extractants, which remove the Co forms which can be taken up by plants (Mitchell, 1964).

(a) Soil extraction

Any soil extractant must remove the forms of a nutrient which are potentially plant available. From Fig. 2.1, it can be seen that for Co these include:

(i) Co in soil solution;

(ii) exchangeable Co present on clay minerals, sesquioxides and organic matter;

(iii) the proportion of adsorbed and occluded Co present on secondary minerals and sesquioxides which is available.

According to Young (1979) various extractants have been used to remove plant available Co and include:

1) 0.01-1.0 M hydrochloric acid.

2) 1 M potassium nitrate at pH 3.

3) 0.1 M calcium chloride.

4) 2% citric acid.

5) 0.2 M oxalic acid.

6) 0.01-0.02 M EDTA.

7) 0.1-1 M nitric acid.

8) ammonium nitrate.

9) 2.5% acetic acid.

Mitchell (1957) found highly significant ($P < 0.001$) correlations between the quantity of available Co removed by either 0.05 M EDTA or 2.5% acetic acid and the herbage Co content of mixed herbage. For historical reasons, Mitchell (1957) suggested acetic acid as the most suitable extractant, with a value of 0.3 mg Co kg^{-1} for a mineral soil at pH 5.5 taken as the borderline value.

Most workers found less Co was extracted by EDTA than acetic acid (Mitchell, 1957; Berrow and Mitchell, 1980; Berrow and Ure, 1985). However, when McLaren et al (1986) used identical extraction time intervals, EDTA removed more Co from soils than acetic acid.

Little information is available on the forms of Co extracted by either EDTA or acetic acid. When Co is extracted with calcium chloride, which extracts soil solution and exchangeable Co, the amounts extracted are very much less than those removed by EDTA and acetic acid (McLaren et al, 1986). It was concluded by McLaren et al (1986) that EDTA and acetic acid extract mainly non-exchangeable forms of Co. From McLaren's work the amount of Co which was plant available was over-estimated by EDTA (as measured after a 16-hour extraction period) and under-estimated by acetic acid for soils with pH <6.0.

Despite these problems, available Co has been traditionally measured by acetic acid in Scotland (MISR/SAC, 1985) and England

and Wales (ADAS/MAFF, 1986). As drainage status has a large influence on Co availability, the data obtained must be interpreted using the drainage class of the soil being examined (Table 2.4). As both pH and organic matter content have major influences on soil Co availability, the data given in Table 2.4 must be adjusted to take account of soil pH and this has been summarised by MISR/SAC (1985).

In some situations, however, acetic acid cannot be used as an indicator of soil Co availability. For instance, when soils are treated with a small amount of water-soluble Co, no detected change has been seen in the amount of Co extracted by acetic acid despite markedly increasing herbage Co contents (Mitchell, 1964). Hence, acetic acid cannot be used as a means of assessing the efficacy of Co pasture treatment as a method of preventing Co deficiency in the grazing ruminant. In addition, acetic acid as a soil extractant, fails to show the sensitivity of Co availability to soil pH (McLaren et al, 1986). For example, a soil adjusted to different pH levels will show identical acetic acid extractable Co concentrations for all pH values, despite herbage Co concentrations decreasing with increased pH. However, when EDTA, a weaker buffered reagent, is used soil pH will influence the amount of Co extracted (Mitchell, 1957).

(b) Prediction of soil Co status using soil series

In recent years, the use of soil trace element maps, as a method of predicting Co status, has attracted some attention. Such maps are based on the trace element analysis of representative profiles within each Soil Survey area mapped by the Soil Survey of Scotland (Berrow, 1986). The primary mapping unit for such maps is the soil series which describes soil type in terms of soil drainage

Table 2.4 Classification and interpretation of extractable soil Co concentrations in relation to animal nutrition [MISR/SAC (1985)].

Soil Co status	Extractable Co (mg kg^{-1}) at pH6 Soil drainage class			Probable herbage Co concentration (mg kg^{-1})
	Free	Imperfect	Poor	
Very low	<0.30	<0.20	<0.20	<0.045
Low	0.30-0.74	0.20-0.65	0.20-0.45	0.045-0.065
Moderate	0.75-1.0	0.66-0.94	0.46-0.85	0.066-0.094
High	>1.0	>0.94	>0.85	>0.094

N.B. For soils containing 12-30% organic matter, the poorly-drained soil data should be used, and for soils of >30% organic matter, herbage Co should be measured.

status and parent material. Berrow (1986) provides the following definitions:

(i) SOIL ASSOCIATION - groups together soils which have developed on the same parent material.

(ii) SOIL SERIES - groups together, within an association, soils of each major soil group (e.g. podzols, gleys, brown forest soils).

As the total trace element content of a soil is determined by the geology of the parent material Co distribution can be related to soil association. However, since the content of the A horizon can be altered by additions from fertilisers, sewage sludges and other waste products, the total Co concentration present in the B horizon may provide a better measure of the inherent Co status of a soil (Berrow, 1986). When the total Co content was analysed for the B horizons of representative samples taken from within a soil association each contained very similar Co concentrations (Berrow, 1987). From such work, it has been possible to classify the total Co content in B horizons as follows (Berrow, 1986):

Low <5 mg Co kg^{-1}

Medium 5-15 mg Co kg^{-1}

High >15 mg Co kg^{-1}

Thus, from the knowledge of which soil association a soil belongs to, it is possible to predict the inherent Co status, but the availability of this Co will be unknown.

In practice, the likelihood of Co deficiency occurring in the grazing ruminant is related to availability of Co in the A horizon, i.e. the main rooting zone. When available Co concentrations in the A horizons of a number of soils within the same association

were sub-divided into their respective soil series, the available Co concentrations found within each series were very similar (Berrow, 1987). As soil series primarily divides soils derived from the same parent material, into groups of differing drainage conditions, such findings would be expected. At the Macaulay Institute, a soil map on the scale 1:50,000 has been produced for the Aberdeen area, delineating the likely occurrence of Co deficiency in terms of soil series. This has agreed with the known distribution of the deficiency in the area (Berrow, 1987). As more details become available it may be possible to produce a number of large-scale maps which can be used to predict the likelihood of Co deficiency in certain areas. However, it must be remembered that such maps take no account of soil pH, which will modify the predicted Co availability in an area. Despite this problem soil trace element maps help to highlight areas of potential Co deficiency.

In a similar manner, COSAC/SARI (1982) have produced tables indicating the likely risk of Co deficiency for soils found in a particular soil series. Using the total Co content of the B horizon of soils, the soil series is classified according to the following groups:

Low risk - over 90% of the fields produce herbage containing adequate Co.

Moderate risk - 50 to 90% of the fields produce herbage containing adequate Co.

High risk - most fields contain herbage containing inadequate Co concentrations.

As with soil mapping, such an approach is only useful as an initial guide to the Co status of an area, and soil and/or herbage analysis should be used to confirm whether there is a risk of Co deficiency.

2.3 HERBAGE FACTORS INFLUENCING COBALT STATUS

2.3.1 The role of Co in the plant

(a) The plant's requirement for Co

To date, it has not been demonstrated conclusively that Co is an essential plant nutrient, although various studies have shown that small additions of Co to the growth medium enhance plant yields. Young (1979) gives a detailed review of the benefits of Co in plant nutrition. To summarise, various workers have found:

(i) Co increases chlorophyll levels and hence improves photosynthesis.

(ii) Addition of Co leads to increased nitrogen and protein levels.

(iii) Levels of various sugars, and starch increase when Co is added.

(iv) Enzyme activity can be altered by adding Co, e.g. additional Co increases catalase and dehydrogenase activity in potatoes, and increases enzyme activity within legume nodules.

(v) Co may lead to increased drought resistance.

Thus, although Co may not be an essential element for most plants, additional Co may be beneficial.

Cobalt is thought to be essential for blue-green algae and many bacteria but not for other algae or higher plants (Salisbury and Ross, 1978). Various workers have demonstrated the Co requirement of different nitrogen-fixing organisms both for growth and the synthesis of vitamin B_{12} like

compounds(Young, 1979). It is required for nitrogen fixation by micro-organisms such as Rhizobium bacteria found in root nodules of legumes and many nonlegumes, but not for normal function of the plant. Delwiche et al (1961) demonstrated conclusively the role of Co in effective Rhizobium symbiosis in Medicago sativa and increasing the Co supply increased dry matter yield by 40% and protein content of legumes, receiving no NO_3-N (Hallsworth and Wilson, 1962). In field experiments, Ozanne et al (1963) found that subterranean clover plots receiving no Co and low fertiliser nitrogen rates remained yellow throughout the growing season. At low nitrogen application rates, Co addition increased yield but at high N-application, Co appeared to be slightly toxic. Their work indicated that, when Co levels in leaves and petioles fell below 0.04 mg Co kg^{-1}, clover yields were reduced.

(b) Uptake of Co by plants

Little information is available on either the forms of Co taken up by plant roots or the mechanisms involved.

In general, workers have concentrated on the role of chelation in plant Co-uptake. For example, DeKock and Mitchell (1957) found the relative amounts of Co in leaves of tomato or mustard plants declined when Co was supplied in a chelated form. They concluded that Co appeared to be more readily absorbed by roots as the simple divalent ions, rather than as an organic complex. From studies using different complexing agents it was suggested that as the residual charge on the chelate increased, uptake was reduced, and since charge appeared to be the one critical factor determining uptake, the entire metallic complex must be absorbed, rather than free Co produced after the complex was broken down (DeKock and Mitchell, 1957). However, the opposite results were obtained for

legumes (Wallace and Mueller, 1983). Chelated Co enhanced Co levels in both stems and leaves, as well as being around twenty times more effective in overcoming Co deficiency in the root nodules than unchelated Co. Hence, legume nodules appear either to have a greater absorbing capacity for chelated Co or the more acidic conditions of the rhizosphere result in organic-complex breakdown releasing Co for uptake.

In addition it has been shown that application of chelated Co to pasture is less effective than cobalt sulphate in increasing herbage Co concentrations (Mitchell and Burridge, 1979; McLaren and Williams, 1981). This must, at least in part, be due to the decreased plant uptake of complexed Co. However, it remains unclear how naturally occurring chelates affect Co uptake.

Little work has been published on the mechanism of Co uptake by plants. Macklon (1986) suggests Co uptake is passive into the root cell cytoplasm, where its concentration is limited by an active movement of Co back into the medium around the roots. Most of the absorbed Co is stored by accumulation in the cell vacuoles within the root, before transport through the shoot. The form in which Co is transported within the plant in unknown. However, a linear relationship has been observed between Co in solution, Co uptake and transport within the plant, irrespective of transpiration rate. Macklon concluded that Co transport would appear to be rigidly controlled.

2.3.2 Plant factors influencing herbage Co concentrations

Most investigations of herbage Co content have concentrated on the influence of sward composition, sward maturity and Co distribution within the plant as determined by sampling strategy.

Various studies have been carried out to establish the relative amount of Co in different grass species. The results obtained are inconsistent but most follow the order quoted by Voss and MacPherson (1977):

Red clover > white clover > Perennial ryegrass > Italian ryegrass > cocksfoot > meadow fescues > timothy.

Different authors have observed that clovers contain higher concentrations than most grasses, with timothy containing the lowest (Hill et al, 1953; Mitchell, 1954; Klessa et al, 1988). In some experiments, clovers have been shown to contain some 100-200% more Co than timothy (Mitchell et al, 1957), while in an area of low soil Co, clover and grass species contained similar amounts (Andrews, 1966). Hill species such as heather and white bent contain relatively high Co contents (Voss and MacPherson, 1977). After reseeding upland pasture, the risk of Co deficiency increases as relatively low Co containing grasses such as timothy and ryegrass replace the natural sward (Voss and MacPherson, 1977).

Although generalised tables giving an ordered list of the relative Co contents of different grass species can be produced, this order will not hold in all cases. Mitchell (1972) in a study to investigate the Co concentrations of ryegrass, cocksfoot and clover at four different sites, found the expected trends at only three of the sites. On the fourth site, cocksfoot contained almost double

the Co content of clover. Mitchell did not give an explanation for this apparent anomaly, but the most likely cause probably lies in the maturity of the plants being different between sites. Thus, to obtain a true comparison of different grass species, care must be taken to ensure all are at identical stages of growth and the same part of the plant is sampled.

As a plant matures the Co concentration changes. For example, Fleming (1970) reported that Co concentration of perennial ryegrass declined with advancing maturity. Similar results were given by Fleming and Murphy (1968) who explained their results in terms of different Co contents of heads, leaves and stems. For grasses Co levels declined in the order:

leaves > flowering heads > stems

In the early stages of growth, the predominance of leafy material will give higher Co contents than later in the season when the proportion of stem material increases. Handreck and Riceman (1969) looked more closely at Co distribution within leaves using radio-active techniques. Their findings showed that Co concentrations were greatest in the margins, base and extreme tips of leaves with Co concentrations in lucerne reaching a maximum soon after emergence and rapidly declining as the leaf expanded.

Many workers have noted seasonal variations in herbage Co, with the highest levels found in late spring and early autumn (Voss and MacPherson, 1977), although such trends are not seen every year. Thus, silage cut in late spring will contain higher Co contents than hay cut in mid-summer (Reith et al, 1983). Such trends can be partly explained by the falling leaf-stem ratio during the growing season, but other factors such as water stress in the summer must contribute to the overall seasonal variation.

In a grazing situation, seasonal variations in Co concentrations are observed in some years but not in others (Barry, 1984). In some cases seasonal variation follows the same pattern as changes in physiological maturity, while in others a higher than average stocking rate may prevent maturation and maintain pasture at an early physiological growth stage. Mitchell and Burridge (1979) carried out experiments to assess the influence of cutting intensity on herbage Co, i.e. plants were maintained at a leafy growth stage. In July, grass was sampled from plots which had never been cut and from plots cut every three weeks. With the exception of clover and timothy, regular cutting maintained herbage Co levels at almost double the concentration of the uncut grass. Voss and MacPherson (1977), however, found lower Co contents in plots which were cut to simulate grazing over the summer months.

The sporadic nature of seasonal variation in herbage Co content can be partly explained by the type of growing season and by stocking rate. In years, where grass growth is abundant or with low stocking rates, grazing animals will not crop the grass to the same extent and maturation can occur. When stocking rate is high the grass will remain at a young growth stage throughout the growing season.

2.3.3 Influence of fertiliser nitrogen application on herbage Co

The influence of fertiliser-N on Co uptake is unclear and the published literature gives a very confused picture. Some workers found no effect of fertiliser-N application on Co content (Stewart and Holmes, 1953; Wright and Lawton, 1954; Mudd, 1970), while others have observed declining Co concentrations when fertiliser-N was applied (Percival et al, 1955; Reith et al,

1964, 1983). On the other hand, Voss and MacPherson (1977) found herbage Co concentrations were increased when fertiliser N was applied and this led to an improvement in the Co status of grazing lambs. Increased herbage Co contents were also obtained after fertiliser N application to a mineral soil (Klessa et al, 1988), and to a peat soil (Reith et al, 1983). Klessa et al (1988) quantified the effect of fertiliser N on a mineral soil by multiple regressions between herbage Co content, soil pH and available soil Co for low and high rates of fertiliser N application during mid and late summer.

The literature provides little clue as to how such conflicting results have arisen. Reith et al (1964) suggested that decreased herbage Co contents may be the result of clover die-back after fertiliser N application and West (1981) proposed a dilution effect of herbage Co when growth rate exceeded the rate of Co uptake. COSAC/SARI (1982) appear to explain the contradictory results in terms of different soil Co levels. They suggest the application of nitrogen reduces Co uptake in soils of deficient or borderline Co status and on naturally freely-drained soils irrespective of Co status. For poorly drained soils with adequate Co contents and on deficient soils treated with Co, the effect of applying nitrogen is variable but generally small (COSAC/SARI, 1982). Klessa et al (1988) suggest that the factors involved are soil availability, acidification by ammonium fertilisers and the ratio of NH_4-N to NO_3-N taken up by roots with respect to the effects on the pH of the rhizosphere.

2.3.4 Assessment of herbage Co contents in relation to the requirement of grazing ruminants

In a natural situation, the only supply of Co to the grazing

ruminant is from the soil via the grazed pasture. Thus, it would appear that a simple analysis to obtain the Co content of the herbage would give a measure of the soil's ability to supply Co to the grazing animal. From the previous sections, it can be seen that although such a procedure is easy to carry out, the results obtained can be difficult to interpret.

Factors such as grass species, their maturity and part of plant eaten during grazing will alter the total Co content of a pasture, and any analysis will represent the amount of Co present in the pasture for that immediate time only (Voss and MacPherson, 1977). To assess the Co status of a pasture, herbage samples must be taken throughout the grazing season.

When grass samples are taken care must be taken to include all the species present in the sward. However, ruminants, and in particular sheep, show selective grazing patterns and certain plant types, although present within the sward, may not necessarily be consumed by the animal. Cornworth (1984) mentions the problems of interpreting herbage data since it is unknown what or how much an animal consumes nor the availability of Co to the rumen microbes from different species or parts of plants. Thus, even with careful sampling techniques the sample obtained may not represent what is actually consumed and metabolised by ruminants.

Despite these difficulties, guidelines have been produced to assess the likely Co status of grazing ruminants, from herbage Co concentrations (COSAC/SARI, 1982):

Deficient - <0.08 mg Co kg DM^{-1}

Borderline - 0.08-0.10 mg Co kg DM^{-1}

Adequate - >0.10 mg Co kg DM^{-1}

These at best can only act as a guide to the likely Co status of the grazing animal and should not be used in isolation as an unequivocal diagnostic method.

Another major problem in collecting herbage samples is soil contamination. As soil contains around a hundred times more Co than the plant, the presence of even minute quantities of soil on plant tissue can result in highly elevated herbage Co concentrations (West, 1981). One method commonly used to detect the presence of soil contamination is to carry out a titanium determination in parallel with the Co analysis. In uncontaminated herbage, titanium levels rarely exceed a few mg kg^{-1} and the presence of >10 mg Ti kg^{-1} is taken as indicating the presence of soil contamination (Fleming, 1965). Other elements, such as iron, are also higher in contaminated samples. Whereas, titanium concentrations are fairly constant in all pasture species, iron is found in higher concentrations in legume species than other grasses (Fleming, 1965). Thus, it is difficult to produce values which would indicate the degree of soil contamination when Fe is used as the indicator.

Whether the presence of any soil adhering to grass samples should be included in a herbage Co determination has been debated. There is a large amount of evidence to support the theory that soil contaminated herbage will lead to an improvement in the Co status of grazing ruminants. Both cattle and sheep can ingest substantial amounts of soil, from 10-100 g soil kg herbage DM^{-1} can be consumed by cattle and up to 300 g for sheep which graze closer to the ground (Thornton, 1983). At least some of the soil Co must be available for rumen microbial synthesis of vitamin B_{12}.

The availability of soil Co to the ruminant was illustrated by early New Zealand workers who used soil drenches as a successful cure of unthriftiness. In later work, Andrews et al (1958) found that heavily grazed pastures appeared to reduce the severity of Co deficiency amoung lambs. Presumably this is a result of greater soil ingestion. In more recent work, Brebner et al (1987) demonstrated that vitamin B_{12} synthesis was greatly enhanced when soil was included in the diet. However, the amounts of soil Co which are available to the rumen microbes are unknown or how this varies in different soil types.

To conclude, at present herbage Co analysis (from uncontaminated samples) can be used only as a guideline to the likely Co status of the grazing ruminant at that immediate point in time when the sample was taken. The development of sampling techniques which can mimic grazing and which will include a certain amount of soil may give a better indication of the Co status of pasture but further work is required to determine the factors influencing soil Co availability to rumen microbes.

2.4 EFFECT OF COBALT ON THE RUMINANT

Cobalt as such is not a physiological requirement of the ruminant, but by various rumen microbes as a precursor to vitamin B_{12} synthesis. Thus, the classic symptoms of Co deficiency such as inappetence, loss of weight and anaemia are a direct result of low vitamin B_{12} synthesis. The role of Co in ruminant nutrition has been reviewed by various authors (Underwood, 1966, 1977; Ammerman, 1970; Robertson, 1981; Gardiner, 1977; Mills, 1981; Clark and Millar, 1983).

2.4.1 Manifestations of Co deficiency

Co does not accumulate in any particular organ or tissue to any large extent, but the liver, kidneys and bones usually contain the highest concentrations (Underwood, 1977). From radioactive studies, Rothery et al (1953) found the highest Co concentrations in the liver, kidney and pancreas. As muscular tissue contains similar amounts of total Co to the liver, Rothery suggested both sites may be important storage organs. These results were confirmed by Smith and Marston (1970) who found vitamin B_{12} was contained in the mitochondria of the liver. They also found that within the gastro-intestinal tract, the tissues of the small intestine contained the highest concentrations of Co.

Before describing the symptoms of Co deficiency it is worthwhile noting the vulnerability of different ruminants to Co deficiency. Most workers agree with Andrews (1971) who found sheep more susceptible than cattle, with the young at most risk.

The simplest way to illustrate the appearance of Co deficiency in ruminants is to follow what occurs once they are subjected to a Co-deficient pasture or fed a Co-depleted diet. Initially, the animals continue to grow and thrive as normal, but after a time-lag of some three to four months for sheep (Stewart, 1951; Andrews, 1955, 1965) or six to ten months for cattle (Gardiner, 1977), the classic symptoms of Co deficiency appear. During this time the animal utilises its reserves of vitamin B_{12} mainly from the liver (Underwood, 1977), the amount stored depending on the previous grazing and feeding pattern. Then follows a period of reduced liveweight gain, and finally loss of weight, severe wasting, anaemia and ultimately death. Other visible symptoms include poor wool production in sheep (Andrews, 1971), dull and

harsh coats in cattle, watery eye discharge which forms a hard crust running down the face, paleness of visible mucous membranes and skin discolouration and fragility (Underwood, 1977). In sheep, increased perinatal mortality in lambs from Co-deficient ewes has been found (Duncan et al, 1981) and lowered lamb viability (Fisher and MacPherson, 1986). The role of Co/vitamin B_{12} on milk production yields of cattle and growth of suckling calves has received some attention. Skerman and O'Halloran (1961) found Co administration to dairy cattle improved the yield of fat-corrected milk and improved milk vitamin B_{12} concentrations by 50% in one herd but had little effect on the other. Heifers receiving Co two months before calving grew better during late pregnancy and lactation than untreated controls. While Co treatment produced no effect on calf birthweight, growth was significantly improved during the first seven weeks (Skerman and O'Halloran, 1962).

On post-mortem of a severely-affected animal, a sad story of extreme emaciation, with often a total absence of body fat is seen. The liver appears fatty with haemosiderisation of the spleen and anaemia is of a normocytic, normochromic type in sheep indicative of bone marrow hypoplasia (Smith et al, 1950; Holmes, 1965; Gawthorne et al, 1966). According to Underwood (1977), inappetence and wasting occur before any signs of anaemia appear and whereas Co administration quickly improves appetite and body weight, the improvement in haemoglobin levels is delayed (Gawthorne et al, 1966). Other changes within the body have been noted. For example, when wethers are maintained on a Co-deficient diet for two and a half years the collagen production in the muscle increases (Holmes, 1965). In addition, cerebrocortical

necrosis in severely deficient wethers has been seen (MacPherson et al, 1976) and Harley et al (1962) found characteristic lesions of polio-encephalo malacia in Co-deficient sheep.

Nowadays severe Co deficiency is rarely seen, occurring normally only under extreme experimental conditions. Of more importance is the existence of sub-clinical Co deficiency where animals fail to show full production potential. As Robertson (1971) explains "sub-clinical deficiency of Co can exist over a large area with farmers unaware of any associated production problem because clinical signs are not apparent and in one district all flocks and herds are more or less simultaneously affected". In addition to a simple lowering of production, the situation can become confounded as animals of sub-clinical Co status show a greater vulnerability to diseases (e.g. parasitic infection) which lowers production further. The extent of the problem is difficult to estimate, with many farmers not even realising they have a problem, and may become more widespread with increased land improvement.

2.4.2 Association of Co with various diseases

The association of Co with various diseases has been observed:

(a) Phalaris staggers and similar diseases

Early workers found that ewes grazing Phalaris tuberosa showed no symptoms of phalaris staggers when given a 7 mg Co drench each week (Lee and Kuckel, 1953). Later work, however, found such rates to be ineffective, with 28 mg Co given weekly or 280 mg Co monthly reducing the incidence of staggers but not giving complete protection, while twice daily dosing with 0.05 mg Co prevented the disease (Lee et al, 1957). The requirement for Co as opposed to vitamin B_{12} was shown by Lee and Kuckel (1957) who suggested that

Co may act by causing proliferation of rumen microbes which can detoxify the active poison in P. aquatica. Timing of Co treatment was found also to be important. For example, administration of Co before putting wethers to graze on a sward containing P. aquatica prevented the development of staggers, but Co treatment after symptoms appeared had no effect (Goddard et al, 1967).

Supplementary Co prevented the development of a similar disease in South Africa (Van der Merwe, 1959) but was ineffective in preventing Helliotropium europaeum poisoning in penned sheep (Lanigan and Whittem, 1970).

(b) Ovine white liver disease

In more recent times an association between the incidence of ovine white liver disease and low Co-status has been observed (Sutherland et al, 1979; Mitchell et al, 1982; McLoughlin et al, 1984, 1986).

(c) Low milk-fat syndrome

The possible prevention of low milk-fat syndrome found in dairy cows when fed a low fibre, high concentrate diet by Co/vitamin B_{12} was first proposed by Frobish and Davis (1977). For three cows, vitamin B_{12} injections increased fat yield by almost 88%, but produced no effect on another four cows. However, Elliot et al (1979), found no difference in milk fat yields when vitamin B_{12} injections were administered to cattle, despite producing increased liver vitamin B_{12} concentrations.

(d) Internal parasitism

Various researchers have found a relationship between Co status and parasitism. In some cases infection with gastro-intestinal parasites resulted in higher worm burdens (Threlkeld et al, 1956), greater worm egg production (Richard et al, 1954) and a greater

loss of weight (Downey, 1966) in Co-supplemented animals. Richard et al (1954) suggested that the additional Co appeared to benefit the parasites rather than the host. However, others (Downey, 1965, 1966; Andrews et al, 1970; MacPherson et al, 1987) found greater mortality and worm burdens in animals of low Co status.

2.4.3 Biochemical consequences of Co deficiency

All the clinical and pathological characteristics of Co deficiency in ruminants result from various metabolic disorders in the tissues.

(a) Enzymic requirement for vitamin B_{12}

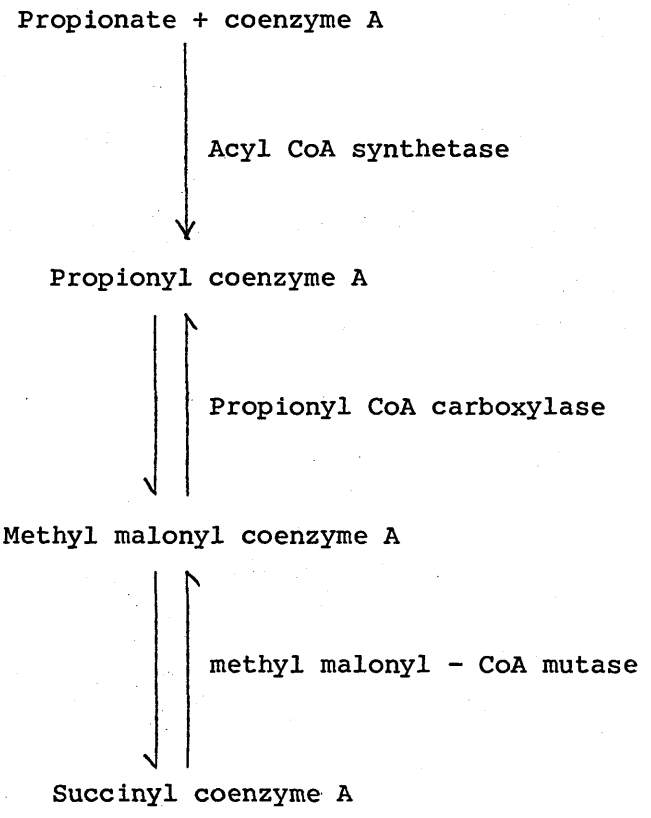
For activity the following enzymes require the presence of vitamin B_{12}:

(i) *Methylmalonyl-CoA mutase (E.C. 5.4.99.2)*

The primary metabolic defect in vitamin B_{12} deficiency is a block in propionic acid breakdown to glucose within the liver (Marston et al, 1961). Propionic acid is a major energy source for ruminants, particularly on high concentrate diets. Initial propionic acid metabolism involves its breakdown to succinyl coenzyme A (Fig. 2.3).

In the early 1970s it was suggested that the impaired utilisation of propionate in vitamin B_{12} deficient sheep might be due to the failure to convert methylmalonyl-CoA to succinyl CoA (Smith and Marston, 1971) and this explained the poor clearance rates of propionate and to a lesser extent acetate in vitamin B_{12} deficient sheep (Somers, 1969). This was further

Figure 2.3 Breakdown of propionic acid to succinyl coenzyme A [from McDonald et al (1981)]



confirmed by Andrews and Hogan (1972) who found increased urine excretion of methyl malonic acid in Co-deficient lambs. When sodium propionate was given to Co-depleted lambs, methyl malonic acid excretion in the urine increased by a factor of ten, but had little effect on Co-supplemented lambs (Hogan et al, 1973), suggesting that the normal propionate pathway was blocked. From results such as these it was concluded that the enzyme methyl malonyl-CoA mutase required vitamin B_{12} as a co-factor. The consequences of impaired propionic acid metabolism can be summarised as follows:

1) Blood propionate levels increase, resulting in a loss of appetite with a consequent lowering of the rate of live-weight gain or an actual loss of weight (Gardiner, 1977).

2) Blood glucose levels decline (MacPherson et al, 1973, 1976).

(ii) *Methyl tetra-hydrofolate-homocysteine transmethylase (E.C. 2.1.1.13)*

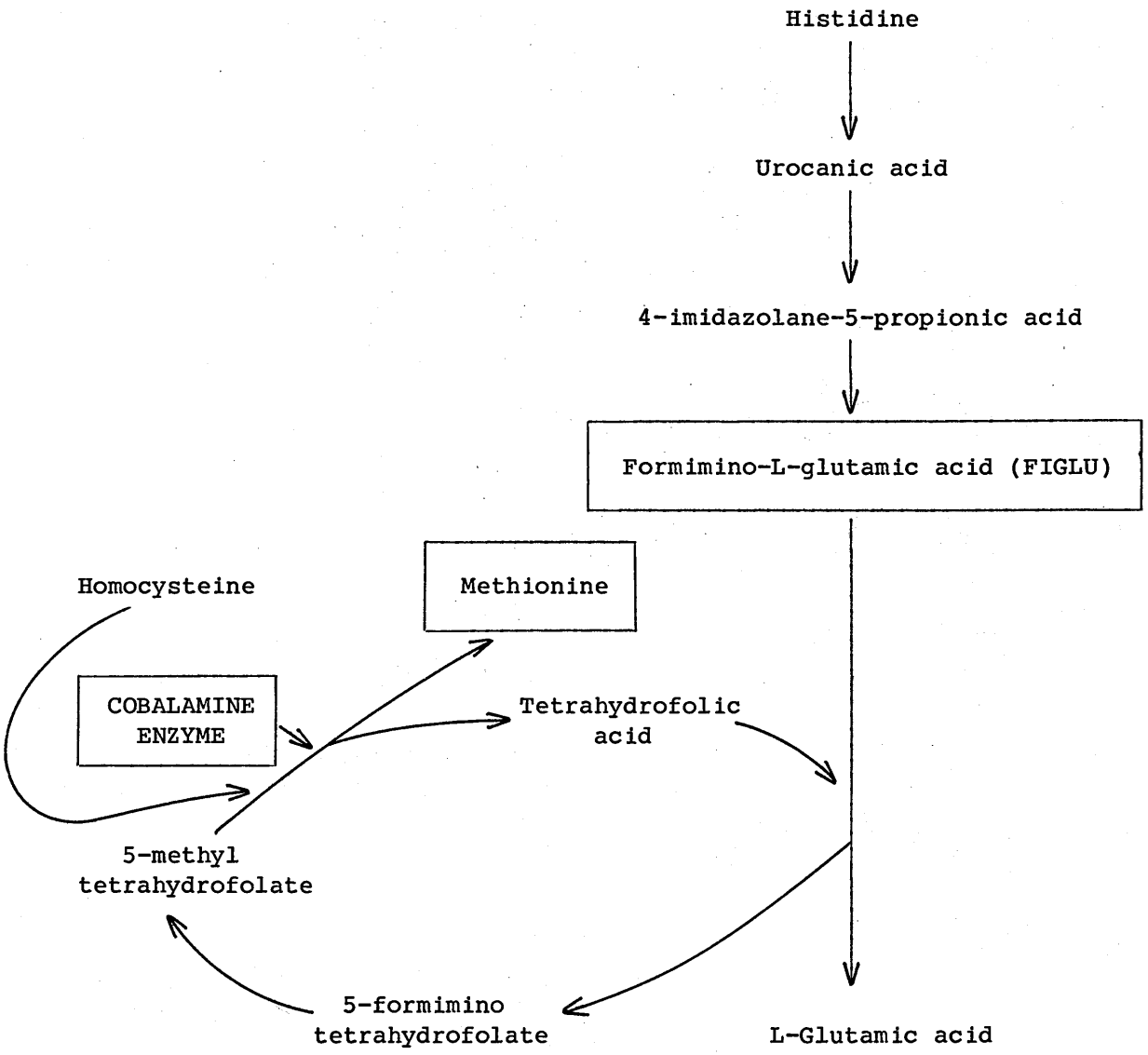
Vitamin B_{12} deficiency has also been found to interfere with the metabolism of folic acid which is another member of the vitamin B group. For many years, it had been observed that vitamin B_{12} or folic acid deficiency in humans resulted in increased excretions of formiminoglutamic acid (FIGLU) but it was not until 1968 that the relationship between vitamin B_{12} and FIGLU was demonstrated in sheep (Gawthorne, 1968). Givens (1978) describes how vitamin B_{12} regulates the availability of tetra-hydrofolic acid, a precursor of folic acid, via the enzyme methyl tetra-hydro-folate-4 homocysteine transmethylase. For the production of folic acid, initial reactions involve the breakdown of the amino acid, histidine,

to FIGLU. For further reactions to take place, tetra-hydrofolic acid is required as a coenzyme. It has been suggested that when vitamin B_{12} is low tetra-hydrofolic acid levels fall in the liver preventing further metabolism of FIGLU which is then excreted in the urine. Smith and Osbourne-White (1973) found very low tetra-hydrofolate and folacin levels in the livers of vitamin B_{12} depleted sheep. In vitamin B_{12} deficient sheep, the activity of both dihydrofolate reductase and 5-methyl tetra-hydrofolate-homocysteine transmethylase in the liver was found to be reduced (Gawthorne and Smith, 1974). As the latter enzyme is required earlier in the reaction scheme to produce tetra-hydrofolate it would appear that this enzyme is the one which requires vitamin B_{12}. Fig. 2.4 gives an outline of the metabolic pathways and where vitamin B_{12} may be involved. Thus, under low vitamin B_{12} status the re-cycling of tetra-hydrofolic acid is reduced, preventing the production of l-glutamic acid, a necessary precursor of folic acid and low folic acid levels were found in livers of vitamin B_{12} deficient sheep (Smith et al, 1974). A deficiency of folacin (collective name for a number of compounds which are derivatives of folic acid) is characterised by nutritional anaemia and poor growth (McDonald et al, 1981). Thus, the characteristic anaemia observed in vitamin B_{12} deficient animals may in part be due to the induced folic acid deficiency.

The consequences of the induced folic acid deficiency can be summarised as follows:

1) From Fig. 2.4 it can be seen that vitamin B_{12} is also required for reformation of the amino acid, methionine.

Figure 2.4 The relationship between Vitamin B_{12} and FIGLU in the 'methionine synthetase' pathway [adapted from Givens (1978)]



As methionine is necessary for optimum growth and wool production, the poor wool growth in sheep and poor coat quality in cattle found with vitamin B_{12} deficiency may be attributed to the fact that the amounts of methionine produced are inadequate. In addition, rumen microbial biosynthesis of methionine is reduced with low Co intake (McDonald et al, 1981). Hence, methionine supply from rumen microbes and synthesis within the ruminant is reduced.

2) DNA synthesis is reduced in Co-deficient animals (McDonald et al, 1981). Various tetrahydrofolates are involved in the synthesis of purines which are important precursors of the various nucleotides present in DNA. In Co deficient animals, the induced folic acid deficiency will result in low amounts of tetrahydrofolates so nucleotide synthesis will be reduced.

3) In Co-deficient sheep, the proportion of lipid material found in the liver increases as choline levels decline (Smith and Osbourne-White, 1974). During choline synthesis, methyl groups are transferred to phosphatidyl ethanolamine by S-adenosyl methionine to produce phosphatidyl choline. Hence, since methionine levels are low in Co deficient animals the methylation reaction no longer occurs (Suttle, 1977) and choline is not synthesised.

(b) Changes in the levels of various blood parameters in severely deficient animals

The concentration of various blood parameters change in severely deficient animals and are summarised as (MacPherson, 1973, 1976):

(i) *Lowered haemoglobin and packed cell volume* due to low haemoglobin synthesis in the bone marrow with a consequent drop in packed cell volume.

(ii) *Liver damage* from increased fat deposition results in aspartate transferase (E.C. 2.3.2.7) leakage into the blood. Plasma ascorbic acid levels decline as synthesis in the liver is impaired.

(iii) *Lower plasma alkaline phosphatase* (E.C. 3.1.3.1) levels occur as a result of the reduced movement of this enzyme, from the gut lining into the bloodstream.

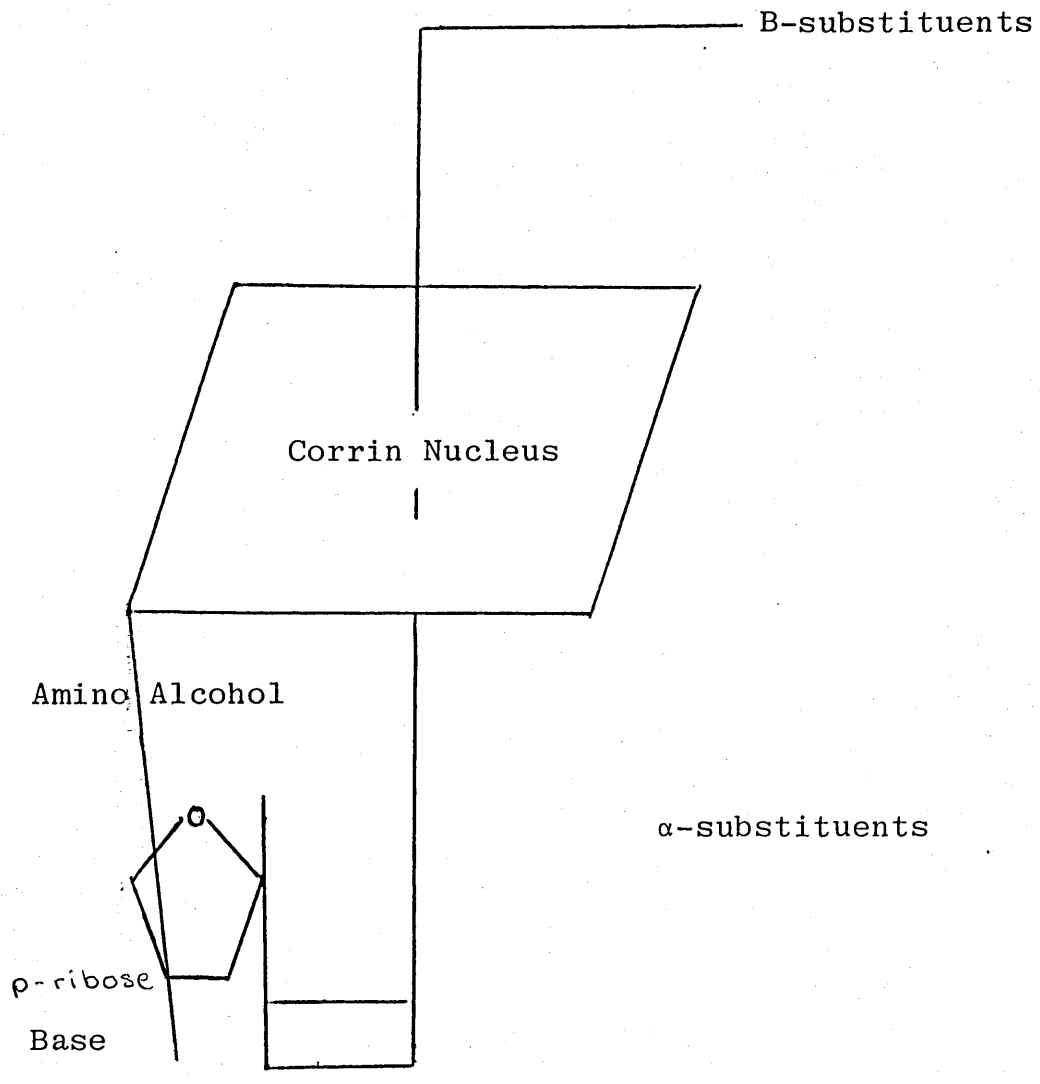
(iv) *Induced thiamin deficiency* causes elevated blood pyruvate and pyruvate kinase (E.C. 2.7.1.40) concentrations. This is thought to arise either from a requirement for vitamin B_{12} by the rumen microbes for thiamin synthesis or the types of rumen micro-flora present changing to a new population which has a lowered capacity for thiamin biosynthesis.

2.4.4 Factors affecting the synthesis of vitamin B_{12}

To date, no one or group of rumen microbes have been isolated as being the main contributors to vitamin B_{12} synthesis. In recent work, using a continuous culture technique, the microbial population appeared to change when a Co-deficient substrate was used, indicating the possible Co dependence (probably as vitamin B_{12}) of some rumen microbes (McDonald and Suttle, 1987). The organisms involved or the amounts of Co required are unknown.

Several forms of vitamin B_{12} are known. In Fig. 2.5 a simplified diagram of the structure of vitamin B_{12} is given. The α-substituents influence the effectiveness by which the rest of the molecule is "identified" by proteins and glycoproteins involved in transport through the intestinal mucosa, transport in the blood-

Figure 2.5 Simplified structure of vitamin B_{12} (from Mills (1980))



stream or through cell membranes, while the β-substituents differ according to the functional role of vitamin B_{12}. So-called "true vitamin B_{12}" has:

α-substituents: amino-propanol, ribose-3-phosphate, 5,6-dimethyl benzimidazole.

β-substituents: methyl (when vitamin B_{12} involved in methylation reactions), adenosyl (for oxidation / reduction reactions).

Vitamin B_{12} molecules with these α-substituents are classed as cobalamins with methyl- and adenosyl-cobalamin the active coenzyme forms of vitamin B_{12} in animals (Mills, 1981).

During microbial synthesis incomplete addition of the various substituents listed above or substitution with other compounds results in a wide variety of cobalamin analogues (Mills, 1981) which are often termed pseudo vitamin B_{12}. Although many of these forms appear to be active in microbes, they have no biological activity within the animal (Gawthorne, 1970; Dryden and Hartman, 1971). This is illustrated in experiments in which injections of pseudo vitamin B_{12} to dairy calves gave no improvement in growth or liver vitamin B_{12} levels, while similar injections with "true" vitamin B_{12} produced a dramatic response (Hopper and Johnson, 1955).

Although Co is the most important dietary constraint on vitamin B_{12} production the nature of the diet fed will alter both the amount of vitamin B_{12} formed and the proportions of inactive analogues. When Co intake is low both total and "true" vitamin B_{12} activity falls in the rumen (Hine and Dawbarn, 1954), with the proportion due to "true" vitamin B_{12} increasing. Smith and Marston (1970) found the efficiency of vitamin B_{12} production

in a basal Co-depleted diet to be 13% compared with 3% when supplementary Co was given and Gawthorne (1970) found as Co intake was increased the proportion in the rumen due to "true" vitamin B_{12} (5,6-dimethyl benzimazolyl cobamide DMBC) declined, while inactive 2-methyl adenylcobamide remained unchanged. He further showed that almost all the vitamin B_{12} activity in the blood of sheep was accounted for as active DMBC. Thus, even although a large proportion (some 60% for Co-depleted sheep) of inactive analogues are produced in the rumen only the active forms are absorbed. With cattle almost 50% of total vitamin B_{12} in plasma could be accounted for as inactive analogues (Halpin et al, 1984).

The nature of the diet fed has been found to influence both overall vitamin B_{12} production and the proportion due to analogues. Roughage restricted diets increased vitamin B_{12} production in cattle (Walker and Elliot, 1972) and sheep (Bigger et al, 1976). High energy rations appear to encourage vitamin B_{12} analogue production in the rumen of cattle at the expense of "true" vitamin B_{12} (Walker and Elliot, 1972). The addition of lucerne meal in the diets of sheep increased production in the rumen of both active and inactive vitamin B_{12} (Bigger et al, 1976) and the proportion of vitamin B_{12} analogues was less in the rumen of grazing sheep than sheep given a roughage-gluten diet (Smith and Marston, 1970). In addition, when food was withheld for some fifty hours, serum vitamin B_{12} levels increased (Millar et al, 1986).

From radio-active labelling studies, Smith and Marston (1970) observed that most of the vitamin B_{12} within the rumen was associated with the micro-organisms but was released in the acid

conditions of the abomasum. Some of the vitamin B_{12} produced in the rumen was degraded and only 5% was absorbed, mainly in the small intestine. Rickard and Elliot (1978) found slightly higher absorption rates than these with between 8 to 38% of the synthesised vitamin being absorbed, and efficiency increasing as the Co content of the diet declined. However, Marston (1970) found absorption efficiencies of vitamin B_{12} of less than 3% for Co-adequate sheep.

2.4.5 Co requirements of the ruminant

Various studies have been carried out in an attempt to find the levels of Co required by ruminants. The minimum total requirement of sheep for vitamin B_{12} has been assessed to be around 11 µg per day to maintain normal growth rates (Smith and Marston, 1970; Marston, 1970), with a dietary Co intake for sheep of 0.08 mg Co day^{-1} (Marston, 1969).

Various workers have suggested minimum Co concentrations which must be present in the diet to maintain adequate Co status. ARC (1982) suggest herbage Co concentrations of 0.11 mg Co kg DM^{-1} as being the lower limit for both sheep and cattle and these values were also initially suggested by Andrews (1965). However, other researchers have found that animals have maintained bodyweight on lower cobalt intakes, and COSAC/SARI suggest 0.07 mg Co kg DM^{-1} as adequate for sheep and around 0.05-0.07 mg Co kg DM^{-1} for cattle. However, to sustain maximum growth rates they suggest that more Co is required at around 0.10 mg Co kg DM^{-1} for sheep and 0.08 mg Co kg DM^{-1} for cattle.

2.4.6/

2.4.6 Measurement of Co status in the ruminant

(a) Liver Co

As the liver is the main storage organ for Co in the ruminant, liver Co levels have been suggested as a diagnostic measure of Co status. McNaught (1948) defined diagnostic criteria, based on a large survey measuring liver Co levels from areas where Co deficiency was unknown, and from Co-treated and untreated animals from known Co-deficient areas:

Sheep	(3 months or older)	<0.06 mg Co kg^{-1}	deficient
		>0.1 mg Co kg^{-1}	adequate
	(under 3 months)	<0.04 mg Co kg^{-1}	deficient
		>0.08 mg Co kg^{-1}	adequate
Cattle	(9 months or older)	<0.05 mg Co kg^{-1}	deficient
		>0.12 mg Co kg^{-1}	adequate

Although data for cattle under 9 months were insufficient to produce any definitive values lower levels than those for older cattle were suggested. Measurements of liver Co levels can, however, be misleading. For example, liver concentrations can be increased in Co-deficient animals showing clinical symptoms by intravenous Co injections or by direct administration of Co into the duodenum without leading to any improvement in the appearance of the animal (Phillipson and Mitchell, 1952). Co supplied in this form is unavailable for rumen microbial vitamin B_{12} synthesis and can be of no benefit to the animals. Also, ruminants kept on a low-Co diet can be maintained free of Co deficiency symptoms by giving regular vitamin B_{12} injections, but produce no effect on liver Co levels (Underwood, 1977). Hence, liver Co levels are not a reliable indicator of Co status.

(b) Liver vitamin B_{12}

Since the metabolically active form of Co is vitamin B_{12}, which is involved in various biochemical processes within the liver, the measurement of liver vitamin B_{12} would appear a more reliable indicator of Co status. Once ruminants are confined to Co-deficient pastures or given a Co-depleted diet, liver vitamin B_{12} reserves become depleted. Dawbarn et al (1963) found over a six month period as Co deficiency progressed mean liver vitamin B_{12} levels fell from 1.05 to 0.12 $\mu g\ g^{-1}$ wet weight. Diagnostic values for liver vitamin B_{12} were suggested by Andrews et al (1960):

	Liver vitamin B_{12} ($\mu g\ g^{-1}$ wet weight)
Very low	<0.07
Low	0.07-0.10
Borderline	0.11-0.19
Normal	>0.19

On supplementation, liver vitamin B_{12} values increase (e.g. Skerman et al, 1959; Andrews and Isaacs, 1964).

However, although liver vitamin B_{12} values provide a reliable measurement of Co status, practical problems prevent its use as a routine diagnostic measurement. Liver samples are both costly and difficult to obtain, requiring either sacrificial killing of a few animals or biopsy samples. Thus, from any one flock the number of animals which can be tested is very limited, making an unequivocal diagnosis of flock Co status difficult.

(c)/

(c) Serum vitamin B_{12}

Vitamin B_{12} concentration in serum is the most commonly used method of determining Co status, mainly as a result of the ease by which samples can be obtained and the low cost involved. Early workers were quick to note the rapid fall in serum vitamin B_{12} concentrations when animals were confined to low Co diets. Dawbarn et al (1957) studied the relationship between plasma vitamin B_{12} levels in sheep with time, when fed diets containing <0.02 mg Co kg DM^{-1}. In one experiment, plasma vitamin B_{12} (as measured by lactobacillus leichmanii) fell to 17% of its original value after 53 days and to only 10% within a further 50 days. In a second experiment, only 2.5% of the initial value was seen after 35 weeks. From their results, Dawbarn et al suggest clinical symptoms of Co deficiency appeared in sheep when plasma vitamin B_{12} values fell below 200 ng l^{-1}. Similar results were obtained by Andrew and Stephenson (1966) who also found clinical symptoms in lambs appearing when serum vitamin B_{12} < 300 ng l^{-1}. Somers and Gawthorne (1969) also observed clinical symptoms in two sheep when plasma vitamin B_{12} levels fell below 200 ng l^{-1} but another two sheep maintained a healthy appearance for a few weeks, even when plasma vitamin B_{12} levels fell below 200 ng l^{-1}. These authors noted the large variation in plasma vitamin B_{12} concentrations within a flock fed an identical ration. This was particularly evident in rations containing greater than 0.10 mg Co kg DM^{-1} and they suggested this was a result of variations in the rate of synthesis of vitamin B_{12} in the rumen and hence the amount present for absorption within the gut. Thus, to assess the Co status of a flock a large number of animals must be sampled. Various diagnostic criteria have been used to classify the Co status of an animal. The values used by the Veterinary Investigational Centres in Scotland are given below (COSAC/SARI):

		Serum vitamin B_{12} concentrations (ng l^{-1}) (measured by lactobacillus leichmanii)
Sheep	Adequate	>400
	Borderline	200-400
	Deficient	<200
Cattle	Adequate	>200
	Deficient	<200

Although these values provide a useful guide to an animal's Co status, they at best provide only an indication of the likely response of animals to treatment. Clark and Millar (1983) describe some of the considerations which must be taken into account when collecting serum samples for vitamin B_{12} analysis such as diseases which can cause liver damage and increase vitamin B_{12} levels and prolonged yarding which increase serum vitamin B_{12} levels. Since serum vitamin B_{12} levels respond within two days of changing dietary Co levels, animals must be sampled while still grazing suspect pasture. Millar et al (1984) found that withholding food for some 44 hours, or supplying only a limited amount of cut grass, increased serum vitamin B_{12} levels. Thus, interpretation of serum vitamin B_{12} values must be made with care. The values used by the Veterinary Investigational Unit will become modified as more data becomes available (Taylor, 1988). Studies to investigate the Co requirements of cattle in particular are scarce and less is known about their requirements. Much of the work to date suggests that much lower diagnostic values should be used for cattle. Mills (1981) suggests a value of <75 ng l^{-1} as indicating Co deficiency in cattle. MacPherson (1981), however, has reported a growth response to Co in cattle at grass where the mean serum vitamin B_{12} concentration of untreated controls was 130 ng l^{-1}.

(d) Urinary or serum methyl malonic acid (MMA)

In more recent years, the potential use of the metabolite MMA either in urine or blood as a method of assessing Co status, has received increasing attention. As discussed earlier, the principal effect of vitamin B_{12} deficiency is to prevent the normal metabolism of propionate, resulting in the build-up of MMA in the blood, and increased excretion in the urine. Since MMA accumulates only once low Co intakes actually influence the ruminant's metabolism, it may relate more closely to loss of production in the animal. Serum vitamin B_{12} levels will fall before the animals' metabolic processes are affected and, thus, before the animal shows any symptoms. Gawthorne (1968) was among the first to note an increased MMA urinary excretion rate in severely Co-deficient sheep of some five to twelve times that of corresponding pair-fed animals given an intramuscular vitamin B_{12} injection. These elevated values could be restored to normal within three weeks of administration of an intramuscular vitamin B_{12} injection. Various other workers have noted higher MMA excretion rates in Co-depleted animals than in supplemented animals (Andrews et al, 1970; Andrews and Hogan, 1972). Definitive values for use diagnostically were proposed by Millar and Lorentz (1979). They suggested that a mean value of >30 $\mu g\ ml^{-1}$ MMA in the urine from ten sheep would indicate Co deficiency in the whole flock. However, the use of urinary MMA values for routine diagnostic purposes is very limited, since specialised equipment is required to obtain a sample. Clark and Millar (1983) state that Animal Health Laboratories in New Zealand do not offer the test because "it is more costly than the liver and serum vitamin B_{12} tests and is no better in detecting Co deficiency".

Within the last five years, sensitive methods of determining MMA in plasma or serum have been developed (McMurray et al, 1986).

Rice et al (1987) suggested normal plasma MMA values for lambs were <5 μmol l^{-1}. They found some 62% of serum vitamin B_{12} values of <250 ng l^{-1} produced MMA values in excess of 5 μmol l^{-1} while the remainder produced low MMA values. They suggest that because of individual animal variation, flock status can only be assessed when a group of animals is tested. At present this work is in the early stages and as more work is carried out the advantages and disadvantages of its use will become more clear.

(e) Urinary formiminoglutamic acid (FIGLU)

As discussed previously (section 2.4.3) FIGLU concentrations are increased in Co-deficient animals as a result of an impairment in tetrahydrofolate production. In pair-fed sheep, severely Co-deficient animals produced around thirty times more FIGLU in the urine than their Co-treated counterparts (Gawthorne, 1968). The high FIGLU levels were detected before urine MMA values increased. Russel et al (1975) studied both serum vitamin B_{12} and FIGLU levels in lambs grazing pasture on soil containing 0.17 mg Co kg^{-1}. After eight weeks, almost half of the lambs showed visual symptoms of Co deficiency, with mean serum vitamin B_{12} values of around 200 ng l^{-1}. FIGLU values were highly variable ranging from 0.08 to 0.55 μmol ml^{-1}, but within one week following Co treatment levels fell to almost zero. These results demonstrate the problems of relating FIGLU levels to the Co status of the animal. However, more recent studies have suggested that FIGLU appears in the urine only in the later stages of Co deficiency when the lambs show visual symptoms of unthriftiness and lose weight (Stebbings and Lewis, 1983). In addition, lambs diagnosed as having sub-clinical Co deficiency produced no FIGLU in the urine while others, classed as being Co-adequate, occasionally produced detectable levels of FIGLU.

Skinner (1983) found similar results, with elevated FIGLU levels in only one flock out of a total of twenty-eight tested, all of which had a history of Co deficiency. Under more controlled experimental conditions, Stebbings and Lewis (1986) found inconsistent FIGLU levels in Co-deficient lambs and occasional high concentrations in one control animal. From this work, FIGLU determinations would appear to be of little benefit in determining the Co status of a flock. However, once the factors controlling its excretion are better understood and detection methods are improved, urinary FIGLU determination may prove to be a successful diagnostic method.

2.5 PREVENTION OF COBALT DEFICIENCY

Cobalt deficiency in ruminants can be prevented provided a regular supply of Co is made available to the rumen microbes since Co storage within the ruminant is limited. Cobalt must be supplied in a form which is available to the rumen microbes, i.e. Co must be ingested rather than injected into the body. Various methods of Co supplementation have been suggested and have met with varying degrees of success.

2.5.1 Application of Co to soils

Cobalt sulphate ($CoSO_4.7H_2O$) either applied as a low volume spray or incorporated into fertiliser has been widely used as a means of ensuring a continuous supply of Co to the grazing animal. Cobalt applied by these methods acts through the soil, leading to increased soil Co availability and greater plant uptake. However, only as little as 4% of the Co added is actually taken up by the plant and, thus, made available to the grazing animal (Nicolls and Honeysett,

1964). According to Underwood (1981) the amount of Co required and the frequency of application depends on the soil type, terrain and husbandry practices used. For instance, a single aerial spray of 0.5 kg Co ha^{-1} applied as hydrated cobalt sulphate every 3-4 years maintained satisfactory herbage concentrations in hill land in New Zealand (Andrews, 1953), while on more accessible sandy soils in Australia, where phosphatic fertilisers are applied at least every second year, as little as 0.1 kg Co ha^{-1} as unhydrated cobalt sulphate every alternative year was found to be adequate (Underwood, 1981).

In Scotland, recommended application rates are 0.6 kg Co ha^{-1} as hydrated cobalt sulphate for an acid Co-deficient soil and 1.2 kg Co ha^{-1} on calcareous soils. These rates were expected to maintain herbage above 0.08 mg Co kg^{-1} DM for a minimum of three years (Reith and Mitchell, 1964), and Reith et al (1983) demonstrated that 0.6 kg Co ha^{-1} was effective for three years on mineral soils and for over five years on peat. However, in more recent times doubt has been cast on whether such application rates are appropriate. For example, McLaren et al (1979) found that even the application of 1.2 kg Co ha^{-1} applied as hydrated cobalt sulphate at one site failed to increase herbage Co concentrations above 0.1 mg Co kg DM^{-1} in the first year. At another two sites herbage Co contents were substantially increased during the year of application but fell to <0.08 mg Co kg DM^{-1} on the plots which received 0.6 kg Co ha^{-1} in the second year. Klessa et al (1988) also found that although the application of 0.6 or 1.2 kg Co ha^{-1} as hydrated cobalt sulphate, significantly increased herbage Co in the year of application, after two years the effect had diminished to such an extent that the herbage could no longer

meet the Co dietary requirements of ruminants. In Wales, Evans (1985) found for a mineral soil applying 0.7 kg Co ha^{-1} as hydrated cobalt sulphate to the seed-bed, elevated herbage Co contents only slightly during the year of application and produced no effect in the second. Higher application rates of 1.45 and 2.87 kg Co ha^{-1} increased herbage levels in the second year following application but had no effect in subsequent years. However, when similar amounts of Co were applied to a peat, residual effects were seen twelve years after application, even for the low application rate (Evans, 1985).

Various workers have attempted to explain the highly variable response of herbage to applied Co. Nicolls and Honeysett (1964) suggested Co uptake from a Co-fertiliser was greater in more acid soils, while Adams et al (1969) stated that the efficiency of Co fertilisers was dependent on the manganese content of the soil. They suggested plants grown on soils containing >1000 mg Mn kg^{-1} total manganese were unlikely to benefit from Co application to the soil. In a recent paper, McLaren et al (1987) demonstrated that plant uptake of added Co to the soil is related to the ability of a soil to sorb Co. Further, he suggests that with the small amounts of Co that are actually added to the soil during Co sulphate application, soil pH will have the overriding influence on Co sorption and hence residual values for cobalt sulphate application.

The recommended rate of 0.6 kg Co ha^{-1} as hydrated cobalt sulphate given by COSAC/SARI should only be taken as a very broad guide-line. Poole et al (1972) suggest the following application rates:

(a)/

(a) Mineral soils (excluding calcareous soils): 0.6 kg Co ha^{-1} as hydrated cobalt sulphate which should last for three years, and they suggest one-third of the grazing area should be treated each year to give a 3-year treatment rotation.

(b) Calcareous soils (those with high pH): larger or more frequent application, and if stocking density is low some other treatment method may be better.

(c) Coastal calcareous sands: due to both the high pH and light texture leading to increased losses, other preventative measures should be used.

(d) Peat soils: generally as in (a) but larger less frequent applications may be used since Co is retained for a longer period.

For mineral soils, consideration must also be given to the manganese content and to a certain extent, iron oxide and clay content (Poole et al, 1972).

Cobalt sulphate can either be applied in solution form or by incorporation into a fertiliser. A Co spray should be applied directly to the soil, before re-seeding or onto short dormant grass in early spring (COSAC/SARI, 1982), to minimise surface contamination of foliage. Co movement within plants is restricted to xylem transport. Hence, although some Co can be adsorbed into the plant via the leaves, this Co will be non-mobile remaining within the leaves. When the grass is eaten, the Co in the leaf will not be replaced and the herbage Co will quickly fall to its original level. By applying Co to the soil a continuous replenishment of Co content of the herbage will take place as Co is

moved up the plant in the xylem (Mengel and Kirby, 1978). Poole et al (1972) mention the use of foliar cobalt sulphate application, where 25 g $CoSO_4 7H_2O$ per fifty lambs is dissolved in water and sprayed onto pasture as a short-term cobalt treatment.

The application of Co during routine fertilisation should be restricted to only manufactured "Cobaltised" fertilisers where Co is distributed throughout the granules. In home-mixed fertilisers it is very difficult to ensure equal distribution throughout.

The use of "hospital plots" on hill farms has been suggested as a means of increasing the Co status of more inaccessible areas (Poole et al, 1972). Under such a system only certain strips of land are treated. However, Voss and MacPherson (1977), suggest such an approach leaves too much to chance, unless a substantial proportion of the grazing area is treated. These authors also point out that continuous grazing of treated pasture is not necessary, and as long as the animals are given access to the treated pasture for 50% of the grazing time, Co deficiency should be prevented.

Various workers have investigated the use of Co-chelates as an alternative soil Co treatment. Most researchers agree that the use of Co-ethylene diaminetetra-acetic acid (Co-EDTA), offers no advantage over treatment with simple salts (Reith and Mitchell, 1964; McLaren and Williams, 1981). Both groups of workers found Co-EDTA addition led to smaller and shorter lasting increases in herbage Co than did cobalt sulphate.

2.5.2 Co Bullet

When grazing is extensive it is frequently impossible and costly to treat the pasture with Co. In such situations Co pellets or bullets can be used (COSAC/SARI, 1982).

Cobalt oxide pellets were first devised by Dewey et al (1958). Using a five gramme mixture containing 90% cobaltic oxide and 10% china clay, small pellets were formed under high temperatures and pressures. The pellet was administered by a balling-gun into the oesophagus, and then became lodged in the reticulum where it slowly dissolved to give a steady supply of Co. From X-ray studies, it was shown that just after the pellet was given, the majority became embedded into the anterior dorsal sac but three months later most were found in the reticulum. In sheep trials, Dewey et al (1958) found such pellets an effective means of reducing the incidence of phalaris staggers and many workers have established the benefits of heavy pellet administration in the prevention of Co deficiency in both sheep and cattle (Andrews, 1958; O'Moore and Smyth, 1958; Andrews et al, 1958; Gracey and Todd, 1959; Skerman et al, 1959; Skerman and O'Halloran, 1962, 1961; Watson et al, 1966). Most workers found the pellet remained an effective source of Co for at least six months.

However, two problems were encountered with this method of treatment - namely regurgitation, and surface coating of the pellet with calcium phosphate which prevents the release of Co from the bullet (Underwood, 1977). Using radio-active pellets (Millar and Andrews, 1964) found almost one-third of the sheep and lambs had lost their pellet after eighteen months. They suggested that most of the pellets were lost by regurgitation rather than lost through the faeces.

Various attempts have been made to reduce the surface encrustation of the pellets with varying degrees of success. The white encrustation was identified as being composed of brushite ($CaHPO_4.2H_2O$) and whitlochite ($Ca_3(PO_4)_2$) and resembled some urinary and salivary calculi and dental tartar (Owen et al, 1968).

Andrews et al (1964) found pre-treatment with alcoholic citric acid did not reduce the formation of calcium phosphate, nor did the administration of two pellets or one pellet plus a screw grinder (Poole and Connolly, 1967). The addition of a grinder did, however, increase the supply of Co from the pellet (Dewey et al, 1969). Connolly and Poole (1967) developed a laminated pellet by wrapping a bandage soaked with cellulose acetate and sprinkled with 5 g cobaltic oxide around a steel rod. Even although such pellets were found to be an effective means of supplying Co without any coating problems, the pellet was never marketed.

In more recent times, a soluble glass bolus impregnated with various trace elements has been developed (U.K. Wellcome Foundation Ltd., 1984). Knott et al (1985) describe the use of a phosphate-based glass bolus, containing Co along with other essential elements. These are administered by a balling gun and become lodged in the reticulum, where the bolus dissolves at a controlled rate to give a continuous supply of Co. The efficacy of such materials containing 0.5% Co as a method of Co supplementation has been shown by Allen et al (1985) and Carlos et al (1985). The recommended yearly dosage rates are:

1 bolus for sheep

2 boluses for cattle

(U.K. Wellcome Foundation Ltd., 1984)

2.5.3 Oral dosing with cobalt sulphate

Supplementary Co must be given frequently since storage within the ruminant is limited, and tissue Co, anyway, cannot enter the rumen where it is required for vitamin B_{12} synthesis (Underwood, 1981). Hence, oral dosing with cobalt sulphate can only be effective if treatment is given regularly at weekly intervals. Dosing sheep twice weekly with 2 mg Co or 7 mg weekly and dosing cattle with 5-10 times these amounts is fully adequate even under severe Co-deficient conditions (Underwood, 1981). As such frequent treatment is not often feasible workers have experimented with larger doses given less frequently. Monthly dosing with 300 mg Co has been found to reduce the severity of Co deficiency but growth remained sub-optimal (Andrews et, 1966). Over a four year period, Lee and Marston (1969) found supplying Co at a rate of 1 mg Co day^{-1} at intervals of three to five weeks prevented the development of clinical Co deficiency, but liveweight gains were lower than those in animals treated once or twice each week. COSAC/SARI (1982) suggested monthly dosages of 250 mg for sheep and 1000 mg for cattle may be an effective method, but not under severe Co-deficient conditions.

As Co is required by the rumen microflora, intravenous injections of 0.1 mg Co daily failed to cure Co-deficient lambs (Phillipson and Mitchell, 1952), while similar amounts given orally led to total disappearance of the symptoms of Co deficiency.

2.5.4 Vitamin B_{12} injections

As with oral cobalt sulphate dosing, vitamin B_{12} injections must be given regularly. The high cost and inconvenience limits the use of injectable vitamin B_{12} to experimental situations (Clark and Millar, 1983). However, even under severely deficient condi-

tions, injections of vitamin B_{12} give a very rapid response. Injections of only 20 µg vitamin B_{12} twice daily for twenty-one days to severely Co-deficient lambs led to increased appetite, and improved liveweight gains within forty to sixty days (Hoekstra et al, 1952). However, after one hundred days, symptoms of Co deficiency re-appeared. Weekly injections of 100 µg vitamin B_{12} prevented Co deficiency (Anderson and Andrews, 1952), but a single dose of 1000 µg resulted in an improvement in condition for only two weeks (Andrews and Anderson, 1954). More recent workers have investigated the use of higher injection rates, given less frequently. For example, 2000 µg vitamin B_{12} boosted mean serum vitamin B_{12} levels of lambs for only three to four weeks (Whitelaw and Russel, 1979), while 750 µg given every three weeks led to an improvement in liveweight gains in lambs (Givens et al, 1979). Under sub-clinical Co deficiency conditions, 1000 µg vitamin B_{12} satisfied the requirement of lambs for fourteen weeks and of weaned wethers for forty weeks (Hannan et al, 1980). Hence, under sub-optimal Co conditions one large injection of vitamin B_{12} may be enough to boost the grazing animals' Co status, but under severe Co deficiency regular injections must be maintained to ensure deficiency symptoms do not develop. The COSAC/SARI (1982) recommended dosage rates are:

250 µg - 1000 µg for sheep

500 µg - 3000 µg for adult cattle

Commercially, two forms of vitamin B_{12} are available. In early experiments, Koch and Smith (1951) found both the hydroxo- and cyano-cobalamin forms were equally effective in correcting cobalt deficiency.

One group of early workers investigated the use of orally administered vitamin B_{12} (Kercher and Smith, 1955) presuming that the ingested vitamin B_{12} would be available for absorption in the same way as vitamin B_{12} synthesised by the rumen microflora although this was not understood at the time. In trials with lambs, 500 µg crystalline vitamin B_{12} given orally each day for five weeks gave a similar response to that from the equivalent amount of vitamin B_{12} given by injection. Smaller amounts of crystalline vitamin B_{12} given by mouth daily for eight weeks was ineffective in preventing the development of Co deficiency. Such a method of supplementation, although effective, would be too costly and require too frequent animal handling for routine treatment. Kercher and Smith (1955) did suggest that the vitamin B_{12} could be added as a feed supplement in the ration. Their results, however, were inconclusive.

2.5.5 Supplementation in the feed

In housed or grazing animals receiving supplementary feeding, cobalt salts or oxides can be incorporated into the ration. COSAC/SARI (1982) recommended mineral mixes containing 100-200 mg Co kg^{-1} Co mixed at a rate of 25 kg $tonne^{-1}$ of concentrates, provided that the concentrate component of the diet forms at least 10% of the final dry matter intake. In Australia, it has become common practice to include some Co in commercial rations irrespective of whether there is any evidence of Co deficiency (Underwood, 1981). As ruminants are highly tolerant of high Co intakes, such levels, although not beneficial, should have no harmful effects.

Salt licks containing 0.1% Co have provided an effective means of eliminating Co deficiency (Underwood, 1981). However,

especially with sheep, their use can be erratic (Gardiner, 1977). Sometimes most of a flock will consume a lick at regular intervals, while other flocks may ignore the lick for days or even weeks and Co deficiency symptoms may appear. Thus, the use of salt licks has largely disappeared as a means of preventing the incidence of cobalt deficiency.

2.5.6 Addition of Co to the water supply

Within the last decade it has become possible to supply Co in the drinking water of housed animals or those at grass which have access to a piped water supply. This method of supplementation is best suited for cattle, who require fairly constant water intakes. By the use of a trace element metering device Co is pumped into the water supply at a concentration of 0.2 to 0.4 mg Co kg H_2O^{-1} (COSAC/SARI, 1982). As the water trough empties, water is replenished drawing in a small amount of concentrated cobalt sulphate solution to maintain a constant Co concentration in the water.

Slow release pellets placed in the filter tube of the water trough have also been tested (MacPherson, 1983). Such pellets slowly dissolve to maintain a constant supply of Co. In trials, with heifers (MacPherson, 1983) either housed or at pasture, such pellets failed to produce any response in the animals' serum vitamin B_{12} levels. The water from such experiments contained only 0.05 to 0.1 mg Co l^{-1} which was found to be an ineffective level of supplementation. Unless new formulations can be produced with faster dissolution rates such a method is unsuitable for the prevention of Co deficiency.

2.5.7/

2.5.7 Addition of Co to anthelmintics

In recent times, anthelmintics containing supplementary Co have been produced. As anthelmintic treatment is repeated every three to four weeks during spring and summer, oral Co can be administered in the anthelmintic drench without any further handling. Since treatment is only carried out at monthly intervals, Co additions to the anthelmintic must be large. Various veterinary pharmaceutical companies have developed anthelmintics containing both Co and Se (Hoechst U.K., 1983, 1984) for use with cattle and sheep. In general, monthly treatment with anthelmintic at the recommended rates will supply around 10 mg Co for sheep and 50 mg Co for cattle. From section 2.5.3 it can be seen that such Co levels are likely to be ineffective in preventing Co deficiency. Little published work is available on the efficacy of this treatment in the field.

2.5.8 Advantages and disadvantages of methods of Co supplementation

The advantages and disadvantages of the various treatment methods are shown in Table 2.5. In most practical situations for long-term treatment the choice of treatment is limited to:

(a) $CoSO_4.7H_2O$ addition to soil, either as a spray or incorporated into a fertiliser.

(b) Cobalt bullet.

If the cobalt content of anthelmintics is increased, this method may become an effective alternative, particularly for sub-clinical deficiency.

Table 2.5 Advantages and disadvantages of different methods of Co supplementation

Method of supplementation	Advantages	Disadvantages
Soil application of $CoSo_4$ $7H_2O$	(a) No handling of animals (b) One treatment should last 1-4 years	(a) In many hill areas, land too inaccessible for application (b) Certain soil types "lock-up" the added Co and reduce availability
Co addition to fertilizers	Requires no extra work, since Co applied with routine fertilizer application	(a) Co must be added at time of manufacture to ensure equal distribution (b) Similar problems of unavailability as with soil applicatons
Cobalt oxide pellets	(a) Requires little handling of animals (b) Can be given to animals, irrespective of terraine	(a) Some pellets are lost by re-gurgitation (b) Coating of pellet with calcium phosphates renders the pellet ineffective
Cobalt glass bolus	(a) Only once a year handling (b) No coating problem due to constant erosion of surface	Some re-gurgitation
Oral dosing with cobalt sulphate	Easy to administer to housed animals	(a) Requires frequent handling - at least weekly or large monthly doses (b) Danger of toxicity if $CoSo_4$ $7H_2O$ is not completely dissolved. Animals treated first will receive a diluted dose, while the final doses will be an almost saturated solution of $CoSo_4$ $7H_2O$

Table 2.5 cont.

Method of supplementation	Advantages	Disadvantages
Vitamin B_{12} injections	Rapid response in severely deficient animals	(a) Requires frequent animal handling (b) Very costly
Supplementation in the feed	No direct animal handling	(a) Must be well incorporated into the feed (b) For grazing animals, difficult to ensure all animals receive adequate amounts
Provision of salt licks	No direct animal handling	Not all animals use the lick at same rate, therefore difficult to ensure every animal receives adequate Co
Addition of cobalt to water supply	No direct animal handling	(a) Care must be taken to ensure Co concentration remains constant (b) Different animals will consume different amounts (c) Consumption varies with rainfall

2.6 COBALT DEFICIENCY UNDER HILL LAND IMPROVEMENT SCHEMES

The risk of Co deficiency, either in clinical or sub-clinical forms has emerged as a problem in areas of improved hill land (COSAC/SARI, 1982). Such reclamation schemes involve drainage and liming to improve soil conditions for the growth of nutritionally better quality grasses and clover (Voss and MacPherson, 1977). Although pedological drainage status has a major influence on Co availability, artificial drainage will probably have a minimal effect, as the processes involved require a long time-scale to make any measurable difference on soil Co. In practice, a combination of liming and the lower contents of reseed grass species are of more importance in reducing the Co status of the pasture to grazing ruminants. In addition, the improved quality of the pasture encourages a more rapid growth rate in lambs and hence increases the animals' requirement for Co. Work carried out by the "Hill Farm Research Organisation" but reported by COSAC/SARI (1982) showed that serum vitamin B_{12} concentrations in ewes and lambs fell below recommended limits when grazing reseeded pastures. Sheep grazing indigenous pasture maintained serum vitamin B_{12} levels of around 800 ng l^{-1}, while those ewes and lambs grazing reseeded pasture produced serum vitamin B_{12} concentrations of 386 and 153 ng l^{-1} respectively. Hence, pasture improvement may induce Co deficiency. Voss and MacPherson (1977) while recognising the problem of induced Co deficiency, suggest pasture improvement should not be discouraged but where a decline in Co status is noted some preventative measure should be taken to counteract the problem.

CHAPTER 3 - ANALYTICAL AND STATISTICAL METHODS

3.1 SAMPLING AND PREPARATION

3.1.1 Soil

Soil samples were taken from the field trial site at Upper Auchinlay Farm (Chapter 9) and the fertiliser N plot trial in 1986 (Chapter 6) to a depth of 15 cm with a step auger using the sampling procedures given by ADAS/MAFF (1980). In 1987, a 10 cm screw auger was used to sample the fertiliser N trial plots. In both years, two samples were taken from each plot and bulked. For the grass species and pH pot trial (Chapter 5), random soil samples were taken from the whole soil volume of each pot using a plastic spoon both at the start and finish of the trial.

All the samples were dried at 35°C overnight or until dry. For both the main field experiment and fertiliser N plot investigation drying was preceded by riddling through a 6.35 mm mesh sieve. After drying only the soils from the main field experiment were milled in a 2 mm rolling mill, the others being broken down manually. All the samples were stored in paper bags and re-dried at 35°C prior to use.

3.1.2 Herbage

Herbage samples were obtained from all the experiments using stainless steel sheep shears cutting at a height of at least 3-5 cm above ground level to minimise soil contamination. In addition, for work at Upper Auchinlay Farm (Chapter 9) samples were also taken using hand operated electric shears ('Perfect Alko 6'). For all experiments except the pot trial, where all the herbage was harvested, representative

samples were obtained at random from the whole experimental area. When this produced a bulked sample of >200 g fresh weight, a sub-sample of around 200 g was removed and retained for analysis.

In all cases the herbage samples were dried at 80-100°C on lined stainless steel trays before further treatment. For samples taken at Upper Auchinlay Farm (Chapters 6 and 9) this involved milling through a 0.6 mm sieve in a "Christie Norris Mill". However, for the grass species and pH pot experiment this procedure would have resulted in insufficient sample being left for analysis. Hence, these samples were manually cut into small fragments using stainless steel scissors.

3.1.3 Animal liveweight measurement

Liveweights were recorded using weightcrushes. For all the sheep trials (Chapters 8 and 9) "Precision Weighers" were used. For work conducted at the Metabolism Unit, West of Scotland College (Chapter 7) an "AMPAC" electronic livestock weigher in conjunction with a cattle crush was used. After moving to the Brickrow Farm Unit, West of Scotland College, this was replaced with a specially made cattle holding pen to which an "Avery" weighing machine was attached.

3.1.4 Faeces and blood collection

Rectum faeces were collected directly from the anal canal using an arm-length plastic glove which when turned inside out and knotted acted as a receptacle. The samples were then stored in the refrigerator until analysed.

Blood samples were collected from the jugular vein using "Vacutainers" with the type used depending on the tests to be performed, i.e.:

(a) SERUM - "Monojet" silicone coated tubes (Sherwood Medical, U.S.A.).

(b) EDTA SERUM - 10 ml "Monojet" tubes as in (a) to which 1 ml 1.5% EDTA/saline solution of pH 6.8 was added.

(c) PLASMA - "Monojet" tubes coated with 143 USP units Lithium Heparin (Sherwood Medical, U.S.A.).

Serum was obtained from clotted blood after retraction in a water bath at 37°C. Plasma containing the protein fibrinogen was separated from the blood cells and platelets by centrifugation at 3000 g for 20 minutes.

Both serum and plasma were stored deep frozen, but all enzymatic determinations were carried out on fresh plasma, within twenty-four hours of collecting the sample.

3.2 SOIL ANALYSIS

3.2.1 Available Co

Available Co was measured as acetic acid extractable Co based on the method of Scott et al (1971). After an overnight initial extraction on an end-over-end shaker with 2.5% acetic acid (1:20 soil to solution ratio), the extract was concentrated by a series of acid evaporation steps to produce a final Co solution which could be read directly on the "Thermo-electron 251" atomic absorption spectrometer at the most sensitive wavelength of 240.7 nm using a deuterium lamp background correction. Sensitivity was ±0.05 μg Co ml^{-1}. All the samples were read against Co standards of 0 to 5 μg Co ml^{-1} containing 0.5% Ca and 0.4% Al.

3.2.2/

3.2.2 Manganese

Soil manganese was determined as both exchangeable and readily reducible Mn by the methods of Scott et al (1971). Exchangeable Mn was determined after shaking overnight with neutral 1 M ammonium acetate (soil to solution ratio of 1:10), while readily reducible Mn was measured on the residue from the ammonium acetate extraction using 0.2% hydroquinone made up in 1 M ammonium acetate. The Mn concentration of each solution was measured directly on a "Thermo-electron 251" atomic absorption spectrometer either at the most sensitive Mn line, 279.4 nm (sensitivity 0.02 μg Mn ml^{-1}) or for concentrations >3 μg Mn ml^{-1} at 403.1 nm line (sensitivity 0.4 μg Mn ml^{-1}).

3.2.3 Soil pH and lime requirement

Soil pH measurement was based on the method of Avery and Bascomb (1974) using a soil to water ratio of 1:2.5. The pH was measured on a "PH M83 Autocal pH meter" (Radiometer), standardised with pH 4 and 7 buffers.

The amount of calcium hydroxide required to obtain a range of pH values (as used in Chapter 5) was obtained by adding 0.001-0.07 g $Ca(OH)_2$ ($\pm$0.0001 g) to 10 cm^3 of soil and measuring the soil pH as above. From a plot of pH against $Ca(OH)_2$ added per cm^3 of soil the quantity of $Ca(OH)_2$ necessary to achieve a range of pH values was determined.

3.2.4 Organic matter and mechanical analysis

Organic matter and mechanical analysis were determined routinely by the West of Scotland College, organic matter being defined as the % loss on ignition after ashing at 680°C according to the method of Avery and Bascomb (1974).

After destruction of organic matter using hydrogen peroxide the particle size distribution was measured by an adapted hydrometer method given by Bouyouces (1951). The particle fractions were defined using the U.K. Soil Texture 1985 system, with all the sand fractions bulked to give a total sand.

3.2.5 Available phosphorus, potassium and magnesium

Available phosphorus, potassium and magnesium were determined routinely by the methods given by MISR/SAC (1985), using a 2-hour shake with 2.5% acetic acid (1:40 soil to solution ratio). Phosphorus was measured colourimetrically by the acidified ammonium molybdate and ascorbic acid method based on the procedure of Murphy and Riley (1962) using a "Vitatron Digital Concentration Photometer" at 880 nm. Potassium was determined directly by flame emission spectroscopy using a potassium filter on a "Corning Flame Photometer" and magnesium by atomic absorption spectroscopy at 285.2 nm (sensitivity 0.1 μg Mg ml^{-1}) on a "Thermo-electron 251" atomic absorption spectrometer.

3.3 PLANT ANALYSIS

3.3.1 Dietary analysis

Dietary analysis of the various feedingstuffs used in the cattle trials (Chapter 7) was carried out routinely at the "Analytical Services Unit", West of Scotland College. Both proteins and the major elements (except sulphur) were determined by a modified Kjeldahl digestion procedure (Spillane, 1966). Total N was measured on the acid extract colourimetrically after the formation of an indophenol blue complex and total Ca, P, Mg, Na and K determined directly

on the extract using an "Inductively Coupled Plasma Spectrometer" (ICP) (Alexander et al, 1985). Sulphur was measured on a nitric/perchloric acid digest with the sulphur content determined turbimetrically (Scott, unpublished). Metabolisable energy evaluation was carried out using the "in vitro" technique of Alexander (1969).

3.3.2 Trace element determination in herbage

(a) Determination of Cu, Zn, Mn and Fe

Cu, Zn, Mn and Fe were determined on an aliquot taken from a dry ash digestion, based on the method of Scott et al (1971). This involved an overnight ashing at 480°C on two successive occasions, followed each time by an acid digestion.

These elements were determined directly using a "Thermo Electron" dual channel sequential scanning ICP. For all determinations, the torch power level was 3 and a pump delay time of fifty seconds with a pump rate of 1.0 ml min^{-1} was used. Multi-element standards and blanks were positioned after every thirty samples in each batch to determine the small calibration drift. The "raw" data was automatically transferred via a RS232 link to a "Macsystem" computer, before proceeding through a simple analogue drift correction programme on a "Comart" micro-computer. The instrumental parameters relating to these elements are given in Table 3.1.

(b) Determinations of Co and Mo

The remaining volume of solution left after the removal of an aliquot for Cu, Zn, Fe and Mn determinations (3.3.2 (a)) was used for Co and Mo analysis. The techniques involved a concentration step to increase the detection limit and separate the trace elements from the major elements such as Mg since it is known that Mo measurement by ICP is subject

Table 3.1 Plasma parameters for Cu, Zn, Fe and Mn

Element	Wavelength (nm)	Calibration concentration ($\mu g\ l^{-1}$)	Torch height (mm)	Integration time (s)
Cu	324.75	5	14	1.0
Zn	213.86	10	14	1.0
Fe	238.20	100	14	1.0
Mn	257.61	20	14	1.0

to spectro interference by Mg (Lyons and Rootayel, 1982). This was achieved by complexation of the trace elements with ammonium pyrrolidine dithiocarbamate at pH 1.0, followed by extraction of the chelated trace elements into chloroform with the organic layer collected by passing through phase separating paper. In order to determine the recovery ratio, Co and Mo standards were also processed through these solvent extraction steps. After separation, the organic fraction was taken to dryness and taken up in dilute hydrochloric acid.

Co and Mo concentrations in solution were determined using an ICP spectrometer using the same general conditions as given for Cu, Zn, Mn and Fe (3.3.2 (a)). The specific parameters relating to Co and Mo are given in Table 3.2. The existence of spectral interference from Fe and Ni on the Co line at 228.61 nm necessitated their determination in each solution so that appropriate correction could be applied. Thus, with each analytical batch three Co standards (0.2 μg Co ml^{-1}) with increasing concentrations of Fe (250, 500, 750 μg Fe ml^{-1}) and three with increasing Ni concentrations (5, 10, 15 μg Ni ml^{-1}) were used to generate the linear correction equations which were then applied by computer calculation to produce a final corrected Co concentration. Further, to ensure the Mo determination was free from Mg interference, the efficacy of the solvent extraction was monitored by measuring the Mg line at 279.55 nm for each solution. As with Cu, Zn, Mn and Fe final calculations were carried out by computer, taking account of the calibration drift.

(c)/

Table 3.2 Plasma parameters for Co and Mo determination by ICP

Element	Wavelength (nm)	Calibration concentration ($\mu g\ l^{-1}$)	Torch height (mm)	Integration time (s)
Co	228.61	0.2	12	10.0
Mo	281.62	2	18	3.0
Fe	234.80	1000	20	2.0
Ni	221.60	10	14	2.0
Mg	279.55	1	14	2.0

(c) Quality Control

Quality control was assessed by the use of control samples. The mean, standard deviation and coefficient of variation (% cv) for the control samples used in 1986 and 1987 are given in Table 3.3. In both years, the % cv was very similar except for Cu, which in 1987 was subject to various analytical problems.

(d) Co and Ti determination by D.C. arc spectrography

In 1986 the herbage obtained at the field site (Chapter 9) was analysed routinely for Co and Ti by D.C. arc spectrography as given by Scott et al (1971).

3.4 ANALYTICAL PROCEDURES USED ON ANIMAL MATERIAL

3.4.1 Serum vitamin B_{12}

Vitamin B_{12} levels in sera were determined routinely by microbiological assay using the method of Taylor and Greer (1982) and the growth medium given by DIFCO (1971). After the precipitation of the serum proteins by boiling with potassium cyanide at pH 4.85, the amount of stable cyanocobalamin present was measured by assessing the growth of the vitamin B_{12} requiring micro-organism lactobacillus leichmannii ATCC 7830 after incubation for 18 to 24 hours at 37°C.

Vitamin B_{12} was also measured by the "Becton Dickinson Vitamin B_{12} (^{57}Co) Radioassay Kit" (Catalogue No. 262315) according to the manufacturers' instructions. Although this kit was originally developed for human sera, it has also been successfully used for ovine samples (Taylor and Greer, 1983). The basic principle of the test is based on "Competitive Protein Binding" in which a known amount of

Table 3.3 Mean tissue element concentration of control herbages measured in 1986 and 1987 (±SD)

Element	Herbage concentration (mg kg^{-1})			
	1986 (n=26)	% c.v.	1987 (n=30)	% c.v.
Co	0.06±0.012	20	0.23±0.045	20
Mo	0.25±0.047	18.8	0.56±0.082	14.6
Fe	58±3.8	6.6	880±39.3	4.5
Mn	173±8.5	4.9	311±10.55	3.4
Zn	23.9±1.80	7.5	37.8±1.50	4.0
Cu	6.17±0.341	5.5	4.37±0.813	18.6

added radio-active vitamin B_{12} competes with unlabelled, endogenous vitamin B_{12} for binding sites on an intrinsic factor, a glycoprotein required for vitamin B_{12} absorption through the gut wall. This particular kit uses "porcine" intrinsic factor which has an equal affinity for both labelled and unlabelled vitamin B_{12} both of which compete for the limited number of available binding sites. Hence, the amount of ^{57}Co which becomes bound will be inversely related to the concentration of vitamin B_{12} in the sample.

3.4.2 Serum methyl malonic acid

Serum methyl malonic acid (MMA) was determined by high resolution capillary gas chromatography as described by McMurray et al (1986). This involved an initial extraction of the MMA into ethylacetate under acidic conditions, followed by conversion to the butyl ester using Butanol/Acetyl chloride. An internal standard, ethyl malonic acid (EMA) was used to compensate for losses and variations accumulated during the analytical procedure.

The concentrations of the MMA and EMA butyl esters present were measured on a "Packard 439" gas chromatographic machine fitted with a 25 x 0.2 mm CP-SIL 5B column (Hewlett-Packard) using a flame-ionisation detector. 2.5 µl of the derivatised sample was injected into the column using a 2-split injection of 10 hexane: 1 sample. The injector port was at a temperature of 200°C with the detector at 280°C. During the chromatography run an oven temperature profile was used, namely:

(i) 120°C for 2 minutes.

(ii) Temperature rise of 5°C min^{-1} until 150°C.

(iii) Rapid temperature rise of 40°C min^{-1} until 230°C.

(iv) 230°C for 5 minutes to flush the column.

A print-out of the peak areas of EMA and MMA was obtained from which the concentration of MMA was obtained.

3.4.3 Neutrophil Function Test

Neutrophil function was determined using a modified candidacidal test as given by Boyne and Arthur (1979). Neutrophils were isolated from EDTA serum by a series of washing and centrifugation steps. The ability of these neutrophils to phagocytose and kill the yeast Candida albicans was measured after an incubation of one hour at 37°C, by utilising the fact that dead Candida albicans stain blue in the presence of the dye methylene blue.

3.4.4 Determination of degree of parasitic infection

Both serum gastrin and pepsinogen were used as blood indices of the degree of parasitic infection (Chapter 7).

Serum gastrin was measured routinely by the "Veterinary Investigational Unit", Ayr, using the "Becton-Dickinson Radioimmunoassay Kit" following the manufacturers' instructions. while serum pepsinogen was determined by the modified colourimetric method of Porter (1977) by the "Veterinary Investigational Unit" at Aberdeen.

From faeces samples, worm egg counts were made using the McMaster flotation technique of ADAS/MAFF Parasitology Manual (undated) for >100 eggs g^{-1} faeces or the Clayton-Lane method as used routinely by the "Veterinary Investigational Unit", Ayr for ≤100 eggs g^{-1} faeces.

3.4.5 Ostertagia ostertagi (stage 3) larvae counts

Infective Ostertagia ostertagi larvae were obtained from cultures maintained at the "Glasgow Veterinary School, Glasgow University"

at a concentration of approximately 15000 larvae ml^{-1} (see Chapter 7). For each weekly batch, the actual amount present was determined using the counting technique of Bairden (1988). Doses were made up by pipetting out an appropriate volume into 20 ml containers and adding around 10 ml water.

3.4.6 Routine analysis of blood

Various blood measurements were monitored throughout the cattle and sheep trials (Chapters 7, 8 and 9). These included total haemoglobin, using Drabkin's reagent as given by "Sigma Diagnostics" Procedure No. 525, serum inorganic phosphorus, plasma glucose, plasma alkaline phosphatase and plasma aspartase transferase using the appropriate colourimetric kit (Sera-Pak Inorganic Phosphorus Kit, Sigma Diagnostic Kit No. 510, Merckotest Alkaline Phosphatase Kit No. 3304 and Sigma Diagnostic Kit No. 505 respectively). In addition, serum urea, and glutathione peroxidase (GSH-Px) concentrations present in whole unclotted blood were carried out routinely by the "Veterinary Investigational Unit", Ayr, using the enzymatic, colourimetric procedure of "BCL Urea Color Kit, Catalogue No. 620235" for urea and an enzyme-coupled colourimetric method based on Paglia and Valentine (1967) and Anderson et al (1978) for GSH-Px. Further plasma Cu, Mg and Ca concentrations were measured by atomic absorption spectroscopy using the routine methods of the "Analytical Services Unit", West of Scotland College.

3.5 STATISTICAL ANALYSIS

All statistical analysis was carried out using statistical computer packages on a Comart computer. This involved "Minitab" (Ryan et al,

1981) for regressions and correlations, while analysis of variance was obtained using "EDEX" (Hunter et al, 1979).

CHAPTER 4 - ADSORPTION AND DESORPTION OF APPLIED COBALT

4.1 INTRODUCTION

The effectiveness of Co treatment of pasture is determined by the ability of a soil to adsorb and retain Co in an available form. Low residual values to Co application have been seen (McLaren et al, 1979;. Reith et al; 1983; Evans, 1985; Klessa et al, 1988), where the increased herbage Co concentrations obtained during the first year disappeared by the second growing season. This has been attributed to irreversible fixation of Co by iron oxides, clay and organic matter (McLaren et al, 1979), but more particularly by manganese containing minerals (McLaren et al, 1979; Evans, 1985). Further, Klessa et al (1988) suggested that the efficacy of pasture treatment may be best defined by an adsorption/desorption index, i.e. the residual value of applied Co may be predicted from soil properties.

Adsorption of Co on to soil components such as magnanese oxides (McKenzie, 1967, 1970; Murray et al, 1968; Loganathan and Burau, 1973; Murray, 1975; Loganathan et al, 1977; Murray and Dillard, 1979; Traina and Doner, 1985), iron oxides (Polgar, 1975; Forbes et al, 1976; Padmanabhan, 1983; McLaren et al, 1986; Borggaard, 1987), clay minerals (Hodgson, 1960; Kabota and Beeson, 1961; Tiller et al, 1963; Kabota and Pendias, 1973; McLaren et al, 1986) and organic matter (Mitchell, 1964; MacCarthy and O'Cinneide, 1974; McLaren et al, 1986) have been studied. However, less work has been carried out with the whole soil to determine the relative importance of these components as Co adsorbing surfaces.

Although Co adsorption has received some attention, little work has been carried out to determine the Co desorption characteristics of

soil. In most sorption studies such as those carried out by Randhawa et al (1985) and McLaren et al (1986), Co desorption has only been a minor component of their work. It is important to note that even if a soil can adsorb large amounts of Co a proportion will remain in a form which is desorbable and, therefore, plant available. Hence, the soil properties determining the amount of adsorbed Co which remains in a desorbable form, may be of more importance in governing the residual value of pasture treatment with Co than those determining the amount of Co adsorbed.

The procedure used to study Co adsorption was very similar to that used by Randhawa et al (1984) and based on the method commonly adopted for phosphate adsorption and desorption. Co adsorption was carried out at a range of Co concentrations calculated to produce equilibrium concentrations in solution which could be measured directly by atomic absorption spectroscopy. In the work of McLaren et al (1986) very much lower Co concentrations as ^{58}Co were used, the concentration being similar to those found naturally in soil solution. However, in the study reported here the use of radio-active isotopes was not possible. In addition, the work was carried out to study the adsorption and desorption properties of Co applied to soil, rather than the behaviour of native Co as investigated by McLaren. However, by adding unlabelled Co no differentation can be made between the Co added and the native Co already present on adsorption sites. Nevertheless, by using high Co concentrations the contribution from native Co will be minimal, as the added Co will be well in excess of natural Co concentrations.

Preliminary experiments were carried out to optimise the procedure to be used and to determine a working range of Co concentrations, and optimise the time period over which adsorption and desorption would be measured.

The aims and objectives of this work were:

(a) To examine Co adsorption characteristics of soils by relating adsorption data to recognised adsorption models.

(b) To study the Co desorption characteristics of soils.

(c) To investigate the soil properties which govern Co adsorption and desorption and from the results produce a series of regression equations which could be used to predict the likely behaviour of soil applied Co.

4.2 MATERIALS AND METHODS

4.2.1 Soil sampling and location

For experiments 1 to 5 a total of fifteen topsoils (0-10 cm) were investigated and these were obtained from various locations (Table 4.1). In Experiment 6, in which the role of pH on Co adsorption and desorption was studied, samples of one soil (soil 16) which had been maintained at different pH values (4.0-8.2) in the field over a number of years was obtained (Table 4.1). Surface samples (0-15 cm) were taken from fallow plots using a trowel.

The relationship between adsorption and desorption to the residual value of Co application was determined for two soils (soils 17 and 18), kindly supplied by C. Evans (ADAS, Trawsgoed, Aberystwyth, Dyfed, Wales) and M. Berrow (Macaulay Institute for Land Use Research, Craigiebuckler, Aberdeen). The locations of these soils are given in Table 4.1.

4.2.2 General analysis

For each soil the following properties were determined by the methods described in Chapter 3:

Table 4.1 Location and properties of soils used in the adsorption and desorption experiments

(a) Location and soil type

Soil no.	Experiment ref.	Location	Grid reference	Soil association	Soil series	Drainage status
1	1,3,4	Bonshaw Farm, Lockerbie	NY242723	Canisbay	Canisbay	Free
2	3,4,5	Glasshouse Investigatonal Unit, W.S.C.	NS383232	Dreghorn	Dreghorn	Free
3	3,4,5	Coylton Grass Plots, W.S.C.	NS379234	Bargour	Bargour	Imperfect
4	3,4,5	Kiln Park, W.S.C.	NS376232	Rowanhill	Caprington	Imperfect
5	3,4,5	Coalfield, W.S.C.	NS375234	Rowanhill	Caprington	Imperfect
6	3,4,5	Kilkerran Wood, Dailly	NS306035	Glenalmond	Glenalmond	Imperfect
7	3,4,5	River Girvan	NX187986	Sand	Sand	Free
8	3,4,5	Doonfoot, Ayr	NS305188	Sand	Sand	Free
9	4,5	Carrick, Ayr	NS308162	Peat	Peat	Poor
10	4,5	Braid Farm, Dailly	NS325015	Peat	Peat	Poor
11	4,5	Upper Auchinlay, Dunblane 1	NN768029	Balrownie	Kippendavie	Imperfect
12	4,5	Upper Auchinlay, Dunblane 2	NN767029	Balrownie	Kippendavie	Imperfect
13	4,5	Upper Auchinlay, Dunblane 3	NN767028	Balrownie	Dunblane	Free
14	4,5	Carrick, Ayr	NS323163	Darleith	Dunlop	Imperfect
15	2,5	Coylton Arable Plots, W.S.C.	NS385235	Bargour	Bargour	Imperfect
16	6	Apiary Plots, W.S.C.	NS398235	Bargour	Bargour	Imperfect
17	7	Pwllpeiran Experimental Farm, Wales	SN774748	Manod	Manod	Free
18	7	Middleton of Blackford, Aberdeen	NJ698355	Foundland	Foundland	Free

(b) Soil properties

Soil No.	pH	% loss on ignition	% sand	% silt	% clay	Exchangeable Mn (mg kg^{-1})	Reducible Mn (mg kg^{-1})
1	5.4	8.5	49.8	31.3	18.9	60.0	118
2	5.2	5.1	77.8	11.3	10.9	7.4	143
3	5.1	10.9	59.1	20.3	20.6	16.2	148
4	6.1	9.6	52.9	23.0	24.0	21.0	84
5	6.1	19.6	47.1	23.7	29.3	7.1	30
6	4.1	8.7	62.1	23.4	14.5	15.1	106
7	5.9	1.9	95.7	4.1	0.2	17.8	101
8	7.9	0.6	91.7	0.8	7.6	11.8	6
9	3.3	94.9	-	-	-	1.2	<0.1
10	3.6	75.2	-	-	-	6.2	3
11	5.4	14.6	38.3	58.6	3.1	31.0	35
12	4.5	20.8	49.8	49.4	0.8	16.8	19
13	6.0	11.3	38.1	30.8	31.1	12.6	156
14	4.2	11.2	48.5	25.4	26.1	9.9	32
15	5.2	8.3	72.4	20.3	14.3	8.3	133
16	4.0 - 8.2	6.8	62.0	17.0	21.0	-	-
17	5.8	16.2	13.4	41.6	45.0	15.0	284
18	6.4	13.1	41.1	49.2	9.8	3.0	21

(a) pH.

(b) Organic matter, as percentage loss on ignition (% LOI).

(c) Mechanical analysis.

(d) Exchangeable and reducible Mn.

Results are given in Table 4.1.

4.2.3 Preliminary Experimentation: Optimisation of experimental conditions for Co adsorption/desorption

(a) Experiment 1 - Determination of the equilibrium time for Co adsorption

Soil 1 was shaken with a Co solution containing 4.42 μg Co ml^{-1} in 0.02 M $CaCl_2$ for different time intervals ranging between 0-168 hours after which the weight of Co adsorbed per unit weight of air-dried soil was calculated.

The influence of shaking time on the amount of Co adsorbed is shown in Table 4.2 and indicated that adsorption was fairly rapid with equilibrium being reached after two hours. Consequently, a two hour shaking time was chosen for all Co adsorption studies. While Randawa et al (1984) agree with this time interval, others (McLaren et al, 1986; Borggaard, 1987) found that between one to seven days was required.

In this experiment, anaerobic conditions developed after shaking for 72 hours. As reducing conditions alter the reactivity of certain soil components, such as hydrous oxides, the Co adsorbing capacity may change from that found under aerobic conditions. Although this problem could have been overcome by aerating the samples with bubbled oxygen, it was felt that since equilibration of the added Co occurred rapidly, a two-hour shake should be adequate for Co adsorption avoiding the development of anaerobic conditions.

Table 4.2 Experiment 1: Influence of shaking time on the amount of Co adsorbed from 4.42 μg Co ml^{-1} solution

Time (hours)	Co sorbed (mg Co/g soil)
0	6.5
1	12.9
2	17.7
4	17.8
6	16.4
25	15.9
30	18.4
72	18.4
78	18.0
96	17.2
144	18.1
168	18.0

(b) Experiment 2 - Determination of an optimum shaking time for Co desorption

The shaking time required for desorption was determined for soil 15 after shaking the soil with a solution containing 14.73 μg Co ml^{-1} in 0.02 M $CaCl_2$. Once adsorption had taken place and the supernatant discarded, the soil pellet was shaken with a Co-free solution (0.02 M $CaCl_2$) for ½-24 hours. The proportion of adsorbed Co which was desorbed (i.e. $\left(\frac{\text{Desorbed Co}}{\text{Adsorbed Co}} \times 100\right)$ was calculated.

The effect of shaking time on % desorption is given in Table 4.3. As with adsorption, desorption was fairly rapid, with around 56% of Co desorbing within the first half hour and equilibrium being reached after four hours. However, a shaking time of two hours was chosen for all the desorption studies, since such a time interval could be more easily fitted into a working day and Co desorption was sufficiently near equilibrium. Similar shaking times were used by Randhawa et al (1984), but McLaren et al (1986) suggested longer intervals of 24 hours.

4.2.4 Co adsorption and desorption methodology

On the basis of the preliminary experiments the following methods were used:

(a) Co adsorption

The method involved weighing 8.00 ± 0.01 g <2 mm air-dried soil into 50 ml polypropylene centrifuge tubes into which 40 ml of a solution containing a known Co concentration was placed. These tubes were shown to have a negligible Co adsorption capacity. The incubating solution was composed of:

Table 4.3 Experiment 2: Influence of shaking time on the amount of Co desorbed after adsorption from a 14.73 μg Co ml^{-1} solution

Time (hours)	% desorption
0.5	55.8
1	58.9
1.5	59.3
2	61.2
4	62.0
24	62.0

(i) 20 ml 0.02 M $CaCl_2$ (analar grade).

(ii) A variable Co concentration derived from a stock solution of 1 mM $CoSO_4$ (analar grade). By pipetting between 0-20 mls of 1 mM, a concentration of 0-29.47 μg Co ml^{-1} was obtained.

(iii) The total 40 ml volume was obtained by adding an appropriate volume of 1 mM calcium sulphate ($CaSO_4 . \frac{1}{2}H_2O$, analar grade).

After a shaking time of two hours (see Experiment 1) at room temperature (20°C), the samples were centrifuged at 4000 rpm for 3 minutes before filtering the supernatant through filter paper (Whatman No. 541) into 20 ml sample cups. The Co remaining in solution was measured by atomic absorption spectroscopy (see section 4.2.5) and the amount of Co sorbed calculated from the difference between the initial incubating medium and the equilibration Co concentration, expressed as Co sorbed g^{-1} soil.

All measurements were carried out in duplicate with a precision of ±2.1%.

(b) Co desorption

Co desorption was studied after adsorption had taken place using the method described in (a). Once the supernatant was discarded, the soil pellet was re-suspended in 40 ml 0.02 M $CaCl_2$ by vigorous hand shaking for 2 minutes. The samples were further shaken for 2 hours on a reciprocating shaker (see Experiment 2) and then centrifuged at 4000 rpm for 3 minutes. Co concentration in solution was measured

by atomic absorption spectroscopy (see section 4.2.5). Desorption was calculated from the amount of Co desorbed into solution, expressed as a percentage of the amount of Co originally adsorbed.

4.2.5 Co analysis

The Co present in solution was determined by atomic adsorption spectroscopy using an "Instrumentation Laboratory Video II aa/ae spectrophotometer" with a wavelength of 252.1 nm, slit width of 80 nm and an oxidising air-acetylene gas supply. At this wavelength sensitivity was 0.1 μg Co ml^{-1}.

4.2.6 Main experiments

Experiment 3 - Characterisation of Co adsorption

The adsorption characteristics of soils 1-8 (Table 4.1) were determined by incubating each with different Co concentrations containing between 0 and 29.47 ug Co ml^{-1}. For soils 1 and 2 an extended isotherm was produced using higher Co concentration of up to 117.88 μg Co ml^{-1} for soil 1 and 58.94 μg Co ml^{-1} for soil 2 using Co stock solutions of 2 and 4 mM $CoSO_4.7H_2O$.

Experiment 4 - An investigation into soil properties influencing Co adsorption

In order to determine the soil properties which are of most importance in determining Co adsorption, the adsorption characteristics of soils 1-14 (Table 4.1) were investigated using solutions containing 0, 2.95, 5.89 and 7.37 μg Co ml^{-1}. The amount of Co adsorbed per unit weight of soil from the 5.89 μg Co ml^{-1} solution was related to soil pH, organic matter, % sand, % silt, % clay and reducible and exchangeable manganese by carrying out both single and multiple regressions. In addition, a similar

regression analysis was carried out using the gradient of the adsorption isotherm or Q/I curve as the dependent variable. For all the soils studied adsorption was linear provided the original Co solution contained <10 μg Co ml^{-1}.

Experiment 5 - Co desorption

(a) Soil properties influencing Co desorption

For soils 1-15, Co desorption was determined as described under 4.2.4 (b) after adsorption from a 14.73 μg ml^{-1} Co solution. During the desorption process, some native as well as applied Co will be desorbed and measured in the final solution. However, in most soils this should be negligible compared with the large amounts of Co added.

(b) Sequential desorption

The objective of this experiment was to determine whether the adsorbed Co was desorbed after further additions of a Co-free solution. In Experiment 5 (a) desorption was determined after only one addition of 0.02 M $CaCl_2$, while in this study, desorption was measured after a further four additions to give a total of five desorption periods over a ten hour interval.

(c) Influence of time on the amount of Co desorbed

After soils 1-15 (Table 4.1) were shaken with a solution containing 14.73 μg Co ml^{-1} which was then decanted after centrifuging, they were oven-dried overnight at 35°C before being left at room temperature for 2, 7, 21 and 28 days before carrying out desorption. Desorption was carried out using only one desorption interval as described in Experiment 5 (a).

Experiment 6 - Influence of pH on Co adsorption and desorption

Soil 16 having a pH of between 4.0-8.2 was used for this work. Adsorption was carried out using an incubating medium containing 14.73 μg Co ml^{-1} and desorption was measured over five sequential periods as detailed in Experiment 5 (b). Desorption was expressed as a percentage of the amount of Co originally adsorbed. Percentage adsorption was calculated as the amount of Co adsorbed from a given addition of Co.

Experiment 7 - Prediction of Co adsorption/desorption characteristics of soil

For soils 17 and 18 the adsorption and desorption characteristics were determined as in Experiments 2 to 5 and compared with the values calculated using various regression equations.

4.3 RESULTS AND DISCUSSION OF EXPERIMENTS

4.3.1 Experiment 3 - Characterisation of Co adsorption

In order to characterise Co adsorption data was applied to various recognised adsorption models, namely the Freundlich, Langmuir and Tempkin isotherms. A brief background to these isotherms is given by Hayward and Trapnell (1964):

(i) *Freundlich Equation*

This equation is commonly presented in the form:

$x = kc^b$

where x is the amount of material adsorbed (μg g^{-1});

c is the equilibrium concentration (μg ml^{-1}) of the final solution and,

k and b (where $b \leqslant 1$) are arbitrary constants.

The Freundlich equation can be linearised to:

$$\log x = \log k + b \log c$$

Therefore, when this model holds, a plot of log x against log c will be linear with a gradient b and intercept log k. Despite k and b having no thermodynamic grounding they can be used for comparing different soils and adsorbing species.

When the Freundlich isotherm holds, the heat of adsorption falls logarithmically with increased surface adsorption. This can result from interactions between adsorbing species, i.e. at low, surface saturation, the adsorbent species can obtain sites far enough away from one another to reduce or prevent interactions but as more becomes adsorbed, interactions are no longer avoidable. With increased adsorption, the distance between adsorbed species becomes less and interactions occur, increasing the energy required for adsorption. Another possible explanation for logarithmic decline in heat of adsorption may lie in the presence of different adsorption sites having different energy levels or affinity for the adsorbate. Initially, low energy sites will be occupied. Few adsorbent species will have enough energy to enter high energy sites, so less adsorption will occur after low energy sites are saturated.

(ii) *Langmuir Equation*

This model was initially developed for adsorption of gases onto surfaces and has been adapted for use in studies of anionic and cationic adsorption by conversion of partial pressures in the original equation to equilibrium concentrations. The normal form of the equation is:

$$x = \frac{pqc}{1 + pc}$$

where x is the amount of material adsorbed ($\mu g\ g^{-1}$);

c is the equilibrium concentration ($\mu g\ ml^{-1}$) as in the Freundlich equation;

q is the maximum adsorption, and

p is the equilibrium constant for the adsorption reaction. This model assumes:

1) the energy of adsorption is constant and independent of surface coverage;

2) adsorption is localised and takes place only on vacant sites, to give a monolayer coverage;

3) maximum adsorption occurs when the surface attains monolayer coverage.

The equation can be linearised to:

$$\frac{1}{x} = \frac{1}{qpc} + \frac{1}{q}$$

For the Langmuir to hold, a plot of $\frac{1}{x}$ against $\frac{1}{c}$ will be a straight line of gradient $\frac{1}{qp}$ and intercept $\frac{1}{q}$.

Whereas the Freundlich equation is purely empirical the Langmuir model can be derived from kinetic, statistical and thermodynamic derivations and has been applied frequently to phosphate adsorption.

(iii) *Tempkin Equation*

This model assumes that the energy of adsorption declines linearly with increased saturation and is described as follows:

$$\theta = \frac{RT}{qo} \log \frac{1 + A_o c}{1 + A_o c e^{-\alpha qo/RT}}$$

where θ is the fraction of surface covered;

R is the ideal gas constant;

T is the temperature;

qo is the heat of adsorption when $\theta = 0$;

A_o is the effective area occupied by the adsorbed molecules;

c is the equilibrium concentration, and

α is a constant.

By ignoring very high and low values of θ the equation can be simplified to:

$$\theta = \frac{RT}{qo} \log A_o c$$

Taking θ to represent x (the amount of Co adsorbed), and $\frac{RT}{qo}$ to be a constant k_1, the Tempkin equation can be expressed as:

$$x = k_1 \log A_o c$$

For the Tempkin model to hold, a plot of x against log c should be a straight line.

The adsorption or $^{Q}/_{I}$ curve was plotted for each soil (Fig. 4.1). These were obtained by plotting the amount of Co adsorbed, i.e. (Q)uantity against the equilibrium concentration, i.e. (I)ntensity. All the soils adsorbed Co to a varying extent ranging from 117.6 μg Co g^{-1} for soil 4 compared to 28.7 μg Co g^{-1} for soil 6.

For all the soils studied curvilinear isotherms were observed similar to those obtained by Tiller et al (1969) and Randhawa et al (1984). At low equilibrium concentrations the gradients

of the isotherms were essentially linear and although a plateau of maximum adsorption was not seen for soils 3 and 8, when higher incubating Co solutions were used for soils 1 and 2 plateaus were observed corresponding to a maximum adsorption of 280 μg Co g^{-1} and 75 μg Co g^{-1} respectively. For Co concentrations containing up to 29.47 μg Co ml^{-1} the isotherm for soils 4, 5 and 6 appeared to be linear, with soils 3, 7 and 8 showing a levelling off at the highest equilibrium concentrations.

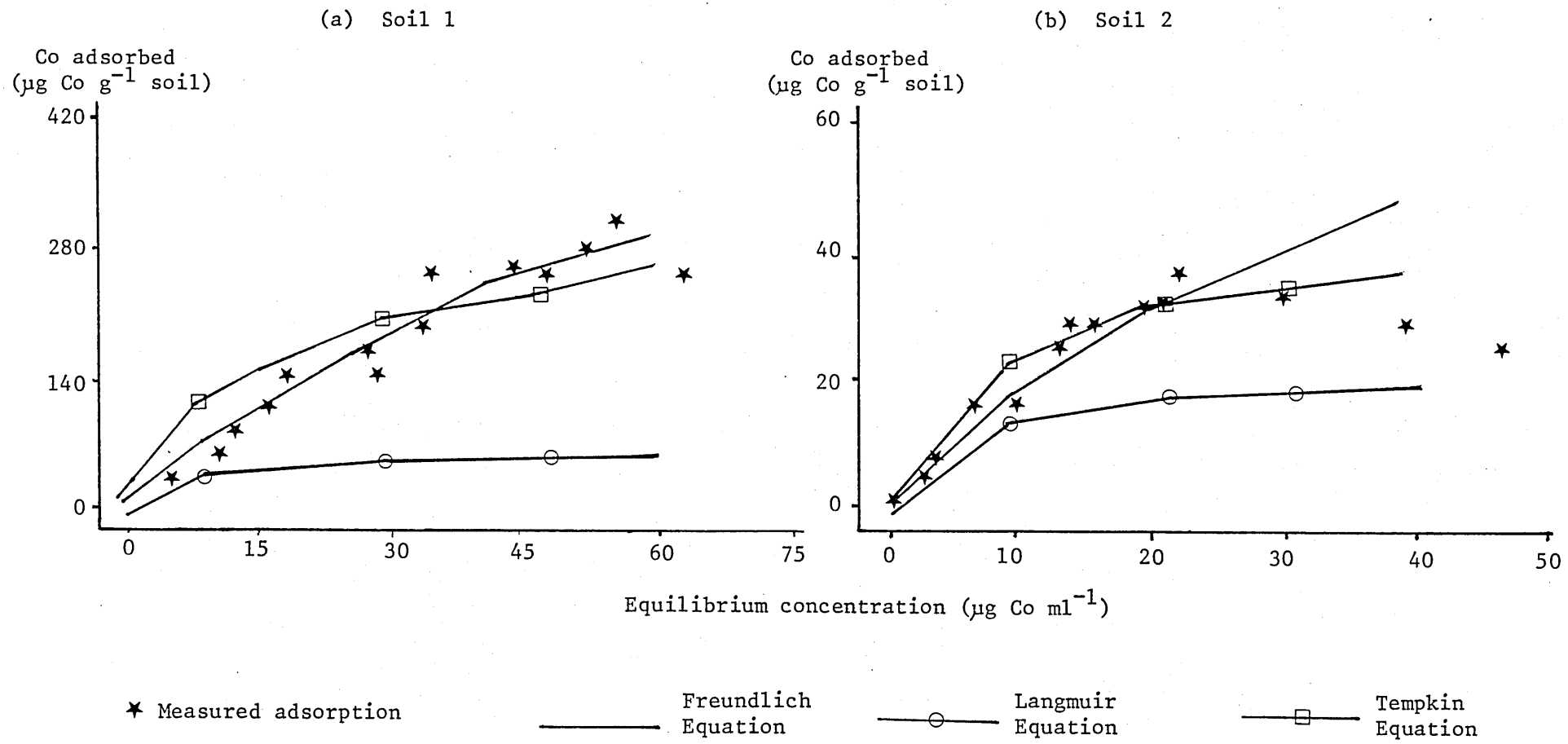
The application of the adsorption data to the linearised forms of the Freundlich, Langmuir and Tempkin isotherm is given in Table 4.4. With each soil, highly significant correlation coefficients were obtained for all three linearised forms of the equations, suggesting that all three can be applied to Co adsorption. Such a situation is impossible since the Langmuir, Freundlich and Tempkin isotherms indicate that the energy of adsorption is independent, or logarithmically dependent or linearly dependent respectively on increased surface coverage. In order to test the models further, plots were made of the predicted adsorption isotherm for the three equations and compared with the measured values (Fig. 4.1). For each equation this involved substituting various values of c (the equilibrium concentration) into the appropriate equation in Table 4.4 to determine the amount of Co adsorbed. In the linear portion of each Q/I curve the measured and calculated values were very similar for each equation. However, for soils 1 and 2 where higher incubating Co solutions were used the Langmuir model predicted adsorbed concentrations of Co in soil well below the actual measured values. For example, for soil 1, the Langmuir equation predicted a maximum adsorption of around 45 μg Co g^{-1}

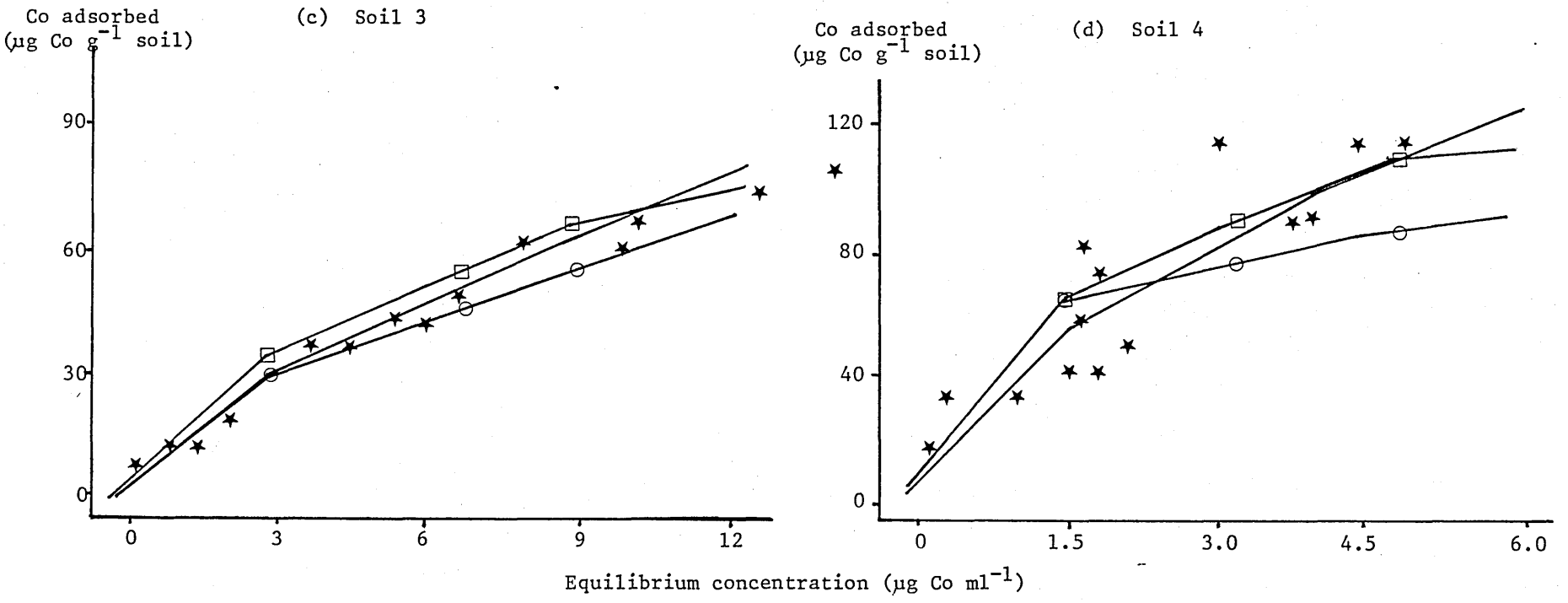
Table 4.4 Experiment 3: Co adsorption by 8 different soils as described by the linearlised forms of the Freundlich, Langmuir and Tempkin isotherms

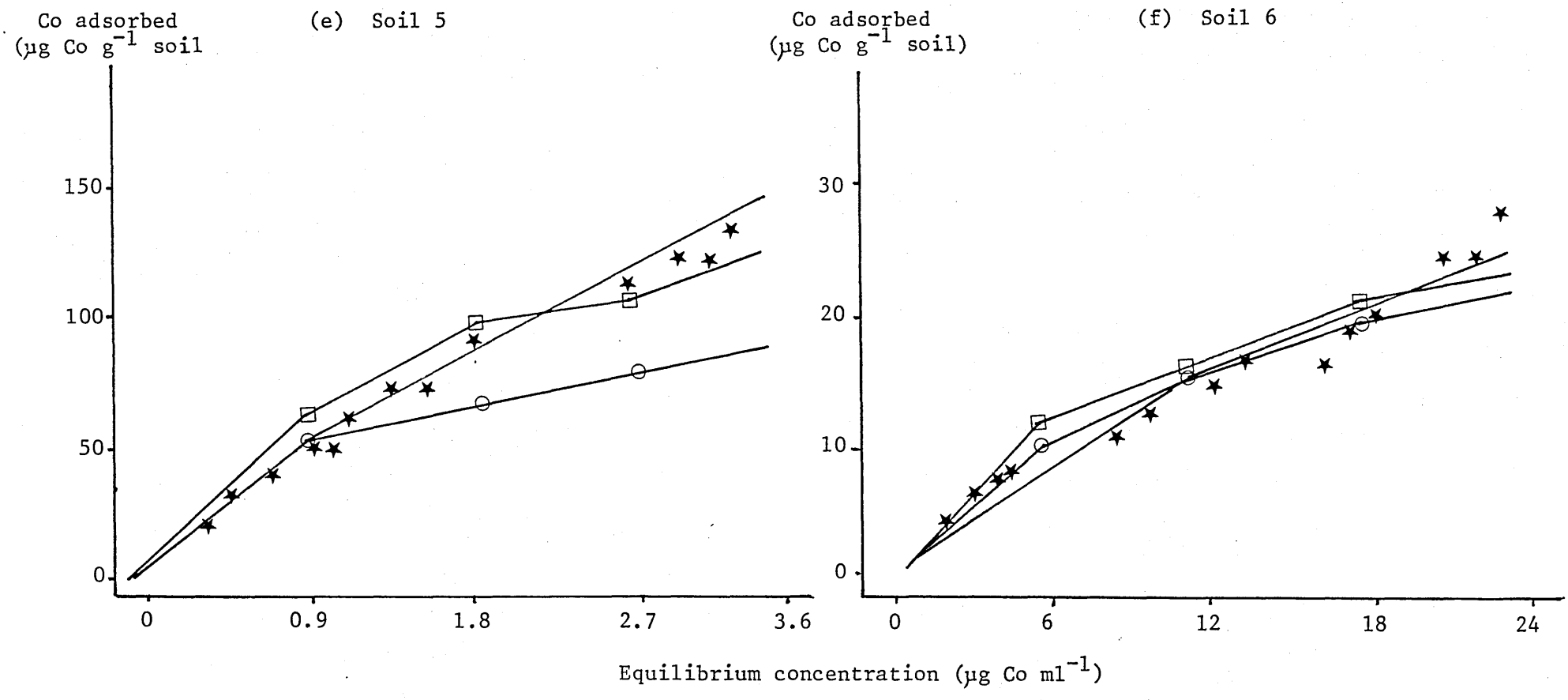
Soil no.	Equation	df	Adsorption equation	SDx	Coefficient correlation
1	Freundlich	37	$\log x = 0.75 \log c + 1.12$	0.080	0.990
	Langmuir		$\frac{1}{x} = \frac{0.03}{c} + 0.02$	0.021	0.958
	Tempkin		$x = 114.99 \log c + 8.69$	46.48	0.877
2	Freundlich	27	$\log x = 0.74 \log c + 0.50$	0.138	0.931
	Langmuir		$\frac{1}{x} = \frac{0.21}{c} + 0.05$	0.104	0.727
	Tempkin		$x = 22.64 \log c + 0.24$	6.546	0.853
3	Freundlich	18	$\log x = 0.75 \log c + 1.06$	0.036	0.993
	Langmuir		$\frac{1}{x} = \frac{0.08}{c} + 0.01$	0.005	0.993
	Tempkin		$x = 51.37 \log c + 9.65$	6.501	0.957
4	Freundlich	17	$\log x = 0.62 \log c + 1.60$	0.107	0.921
	Langmuir		$\frac{1}{x} = \frac{0.01}{c} + 0.01$	0.007	0.898
	Tempkin		$x = 71.39 \log c + 48.84$	17.49	0.857
5	Freundlich	18	$\log x = 0.74 \log c + 1.73$	0.039	0.993
	Langmuir		$\frac{1}{x} = \frac{0.01}{c} + 0.01$	0.008	0.971
	Tempkin		$x = 77.04 \log c + 68.13$	14.27	0.928
6	Freundlich	18	$\log x = 0.73 \log c + 0.38$	0.038	0.990
	Langmuir		$\frac{1}{x} = \frac{0.39}{c} + 0.04$	0.012	0.990
	Tempkin		$x = 18.11 \log c - 2.99$	3.226	0.903
7	Freundlich	18	$\log x = 0.69 \log c + 0.76$	0.022	0.997
	Langmuir		$\frac{1}{x} = \frac{0.16}{c} + 0.02$	0.005	0.995
	Tempkin		$x = 29.36 \log c + 1.08$	3.909	0.950
8	Freundlich	17	$\log x = 0.45 \log c + 1.31$	0.026	0.992
	Langmuir		$\frac{1}{x} = \frac{0.03}{c} + 0.02$	0.003	0.983
	Tempkin		$x = 36.80 \log c + 20.10$	3.547	0.978

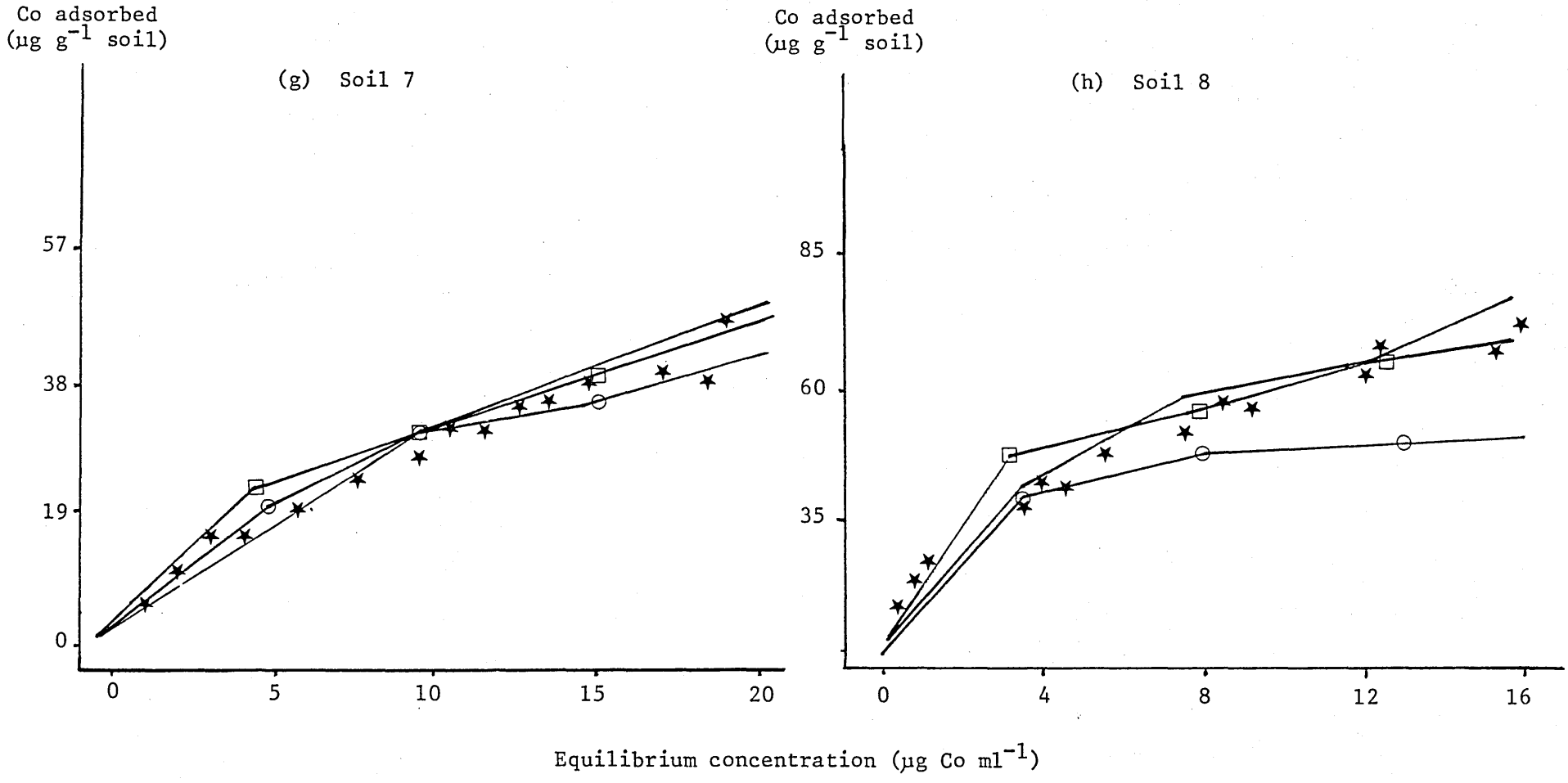
N.B. All the correlation coefficients were significant at $P<0.001$.

Figure 4.1 Relationship between the Freundlich, Langmuir and Tempkin Isotherms to measured Co adsorption




Co adsorbed
(µg Co g^{-1} soil)
(c) Soil 3
90
60
30
0
0
3
6
9
12
Co adsorbed
(µg Co g^{-1} soil)
(d) Soil 4
120
80
40
0
0
1.5
3.0
4.5
6.0
Equilibrium concentration (µg Co ml^{-1})


(e) Soil 5
Co adsorbed
(µg Co g^{-1} soil
150
100
50
0
0
0.9
1.8
2.7
3.6
(f) Soil 6
Co adsorbed
(µg Co g^{-1} soil)
30
20
10
0
0
6
12
18
24
Equilibrium concentration (µg Co ml^{-1})


Co adsorbed
(µg g^{-1} soil)
(g) Soil 7
57
38
19
0
0
5
10
15
20
Co adsorbed
(µg g^{-1} soil)
(h) Soil 8
85
60
35
0
4
8
12
16
Equilibrium concentration (µg Co ml^{-1})

while the measured value was in excess of 280 ug Co g^{-1}. A similar but smaller difference was seen for soil 2. In contrast, both the Freundlich and Tempkin equations produced isotherms similar to the measured values. For soils 3 and 8 where maximum adsorption was not obtained, all three isotherms provided good agreement between predicted and measured values. In each case, the Langmuir model underestimated the amount of Co adsorbed at high equilibrium concentrations, with the Freundlich and Tempkin models more closely following measured adsorption. Hence, although the correlation coefficients suggested that both the Freundlich and Langmuir isotherms described Co adsorption equally well, when the predicted isotherm was compared with measured values, the Freundlich model produced the "best fit". On the other hand, while the Tempkin model gave the lowest correlation coefficients, when the predicted and measured isotherms were compared, good agreement was seen. From the data available, no differentiation could be made between the "goodness of fit" of the Freundlich and Tempkin isotherms, with both models predicting the adsorption characteristics of Co reasonably well.

In all previous studies, only the Langmuir model has been applied to Co adsorption (Tiller et al, 1969; Randhawa et al, 1984). As with this study, significant linear regressions were obtained, but no attempt was made by Tiller or Randhawa to check the validity of their work by comparing predicted with measured values. From the results given here, the Langmuir model did hold at low equilibrium concentrations. Hence, at the low site coverage examined by Tiller it is not surprising that the adsorption of Co predicted by the Langmuir isotherm followed the observed pattern. Hayward and Trapnell (1964) stated that as a

matter of principle to test the fit of data to one particular adsorption model a large concentration range should be used.

In this study, both the Freundlich and Tempkin models appeared to give the best fit to the data suggesting that the energy of adsorption declines with increased surface coverage. This is normally due to interactions between adsorbed and adsorbing ions and can be of two types:

(a) Charge repulsion in which the approaching ion is repelled by the adsorbed ion.

(b) Size exclusion where the physical size of the adsorbed ion prevents another ion approaching a vacant site.

As little is known about the form in which Co is adsorbed, it is difficult to assess which of these mechanisms is operative. In a detailed study of Co adsorption using montmorillonite, Hodgson et al (1964) suggested that no charge was associated with Co once it was adsorbed. If this is the case for all adsorbing surfaces, the size exclusion factor may best explain the decline in energy of adsorption with increased surface saturation.

On close examination of the Freundlich equations obtained for soils 1 to 7 (Table 4.4), it can be seen that the gradient (b) was similar in all cases (Mean = 0.72 $\pm$ 0.03 S.D.). This suggests that the adsorption mechanisms involved in each of these soils was similar. Co adsorption is known to occur onto various soil components (e.g. organic matter, clays and hydrous oxides) and it is unknown whether each uses the same process. However, if Co adsorption is dominated by one soil constituent, the overall mechanism will be similar for each soil. On examination of Table 4.1, it can be seen that soils 1 to 7 contain very much

higher reducible manganese contents than soil 8 which produced a very much lower value of b (0.45), with all other soil properties being similar. This suggests that manganese may be important in determining the mechanism of Co adsorption in the soil. However, reducible manganese only measures a fraction of the total manganese present in a soil and gives no indication of the forms or concentrations of manganese minerals present.

4.3.2 Experiment 4 - An investigation into soil properties influencing Co adsorption

(a) Correlation between soil properties and the amount of Co adsorbed

The amount of Co adsorbed from 5.89 μg Co ml^{-1} solution was related to soil pH, organic matter (% loss on ignition), % sand, % silt, % clay, manganese (exchangeable and reducible) by carrying out both single and multiple regressions. Only the mineral soils were used in the regression analysis, with the two peats (soils 9 and 10) excluded. Those correlations which were significant are shown in Table 4.5. Soil pH was the only single property which significantly ($P < 0.05\%$) influenced Co adsorption, with increased pH resulting in greater amounts of Co being adsorbed. The correlation coefficients were further improved by combinations of:

(i) pH, % sand

(ii) pH, % loss on ignition

(iii) pH, % silt

but multiple regressions of the combinations of pH, % loss on ignition, % sand (or % clay) failed to increase the correlation coefficients significantly.

Table 4.5 Experiment 4: Soil properties giving significant correlations when regressed against Co adsorption (y) from an initial solution of 5.89 μg Co ml^{-1}

Soil property	Degrees of freedom	Regression equation	SDy	Correlation coefficient (r)	t
pH	10	y = 4.62 pH - 6.70	5.760	0.62*	
pH, % loss on ignition (LOI)	9	y = 6.49 pH + 0.77% loss on ignition - 24.84	3.746	0.86**	t_{pH} = 5.52*** t_{LOI} = 3.83**
pH, % sand	9	y = 6.50 pH - 0.27% sand - 1.28	3.102	0.91**	t_{pH} = 6.76*** t_{sand} = -5.50***
pH, % silt	9	y = 6.45 pH + 0.26% silt - 23.30	4.303	0.81**	t_{pH} = 4.70** t_{silt} = 2.99*

Significance of correlation coefficient and t value

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

The strong influence of soil pH on Co adsorption has been observed by others (Tiller et al, 1969; Padmanabham, 1983; McLaren et al, 1986; Borggard, 1987; Anderson and Christensen, 1988) and has been attributed to the pH-dependent adsorption capacities of the clay minerals (Hodgson, 1960), goethite (Padmanabham, 1983), hydrous manganese oxides (Loganathan et al, 1977). As pH rises the negative charge present on pH-dependent charged surfaces increases (Wild, 1988) and, therefore, leads to a greater number of potential adsorbing sites.

No single soil component is highlighted as showing a strong affinity for Co. When the amount of Co adsorbed was correlated with the various soil components positive (non-significant) correlation coefficients were obtained with % organic matter (as % loss on ignition), % silt, % clay and Mn (exchangeable) and negative (non-significant) correlation coefficients with % sand and Mn (reducible). Hence, as the amounts of organic matter, silt and clay increase, more Co is adsorbed. However, soil is a highly complex matrix made up of various organic and mineral components in close association, making it difficult to attribute Co adsorption to any one component. For example, Hodgson (1969) found Co adsorption on to clay was greatly enhanced after the iron oxide coatings were removed. Therefore, even if an isolated soil constituent is shown to adsorb Co, when present in soil the various interactions which take place between the different components are likely to alter its reactivity to Co.

In this study only exchangeable and reducible manganese were measured, no account being taken of either total manganese or iron content. Borggaard (1987) found no significant correlations between Co adsorption and EDTA extractable manganese. However, when total

Manganese was used, McKenzie (1967) found a strong relationship between Mn and Co adsorption. Since total Mn was not measured in this study, no conclusions can be made regarding the role of Mn minerals except that the Mn content measured as exchangeable or reducible Mn had no effect on Co adsorption. In the work of Borggaard (1987) a strong association was shown between Co adsorption and the iron oxide fraction, and McLaren et al (1986) suggested that the soil oxide fraction showed the strongest affinity for Co.

As shown by other workers (Tiller et al, 1969; Borggaard, 1987; Anderson and Christensen, 1988) non-significant correlation coefficients were obtained when % loss on ignition was regressed against Co adsorbed, despite the fairly large adsorption capacities of the two peats (soils 9 and 10) (Table 4.6).

No significant correlation coefficients were obtained between Co adsorption and clay content, despite clay having been shown to have a strong affinity for Co (Hodgson, 1960). However, various clay minerals show different affinities for Co (Tiller et al, 1963) and their relative proportions will govern Co adsorption. In addition, the adsorption sites present on clay surfaces are masked by various other components such as hydrous oxides, and organic matter and adsorption sites on clays may become inaccessible to Co. Indeed McLaren et al (1986) suggested that clays may be of minor importance in Co adsorption and this certainly appears to be the case in this work. While Randhawa (1984) did find a significant relationship between clay content and Co adsorption, this may have been due to oxide and organic matter coatings on the clay surface.

(b)/

Table 4.6 Experiment 4: The amount of Co adsorbed by the various soils from a 5.89 μg Co ml^{-1} solution

Soil No.	Co adsorbed (μg Co g^{-1} soil)
1	22.7
2	8.1
3	18.6
4	28.0
5	27.2
6	8.1
7	13.7
8	23.9
9	19.3
10	19.8
11	21.2
12	17.9
13	25.4
14	9.2
15	15.8

(b) Correlations between soil properties and the gradient of the Q/I curve

When the gradient of the Q/I curve was regressed against various soil properties no single property gave a significant correlation coefficient as was also found by McLaren et al (1986). For multiple regression, significant correlation coefficients were obtained between gradient versus % loss on ignition and pH, and gradient versus % sand and pH (Table 4.7). However, further multiple regression of pH, % loss on ignition, % sand versus gradient gave no improvement in the correlation coefficient, while the combination of pH, % loss on ignition and % clay produced the highest correlation coefficients and significantly improved explained variance over that of pH, % loss on ignition and pH, % clay, despite the latter multiple regression giving a non-significant correlation coefficient. Various other workers have shown the influence of clay on the gradient of the Q/I curve, but found no effect of organic matter (Tiller et al, 1969; Randhawa et al, 1984). The two peats (soils 9 and 10) gave fairly high values for the gradient (Table 4.8), with soil 9, which had the highest % loss on ignition, giving higher values than soil 10. No relationship was observed between Mn (as exchangeable or reducible) and the gradient of the Q/I curve. However, total Mn has been shown to significantly influence the gradient (Tiller et al, 1969; Randhawa et al, 1984). Hence, although exchangeable and reducible Mn do not affect the gradient, the influence of Mn cannot be dismissed.

The gradient of the Q/I curve gives a measure of the buffering capacity of a soil, i.e. as soil solution concentration falls Co is released from adsorption sites, to replenish the supply. Such a measure of Co adsorption is more useful than using one adsorption point on the adsorption isotherm, but checks must be made to ensure the data points used do correspond to the linear portion.

Table 4.7 Experiment 4: Soil properties giving significant correlations when regressed against the gradient of the $^{Q}/_{I}$ curve (y)

Soil property	Degrees of freedom	Regression equation	SDy	Correlation coefficient (r)	t
pH, % loss on ignition	9	y = 10.12 pH + 1.85% loss on ignition - 61.31	9.169	0.78*	$t_{pH} = 3.52^{**}$ $t_{LOI} = 3.73^{**}$
pH, % sand	9	y = 9.52 pH - 0.54% sand - 6.86	9.966	0.73*	$t_{pH} = 3.08^{*}$ $t_{sand} = -3.23$
pH, % loss on ignition, % clay	8	y = 9.48 pH + 1.57% loss on ignition + 0.56% clay - 63.75	6.707	0.89**	$t_{pH} = 4.48^{**}$ $t_{LOI} = 4.28^{**}$ $t_{clay} = 2.97^{*}$

Significance of correlation coefficient and t value

*P<0.05; **P<0.01

Table 4.8 Experiment 4: Gradient of the Q/I curve for the various soils

Soil No.	Gradient of the Q/I curve
1	9.41
2	2.09
3	7.70
4	17.30
5	49.02
6	0.94
7	3.25
8	10.35
9	14.86
10	6.28
11	33.36
12	2.27

It has been suggested (Klessa et al, 1988) that the efficacy of Co treatment could be measured in terms of an adsorption/desorption index. From a regression equation relating the soil properties to Co adsorption it should be possible to predict what will occur when Co is applied. As the gradient of the $Q/_I$ curve is more universally applicable than the amount of Co adsorbed from a single Co solution of known concentration, the former would appear to be the best measurement to use in helping to predict the residual value of applied Co to pasture.

4.3.3 Experiment 5 - Co desorption

(a) Soil properties influencing Co desorption

The % desorption values obtained for soils 1-15 are given in Table 4.9. These were then regressed against soil properties (Table 4.10). Both organic matter and soil texture influenced % desorption but pH was the most important factor governing the degree of desorption.

(b) Sequential Co desorption

The objective of this experiment was to mimic in an accelerated fashion what occurs in the soil situation where plant uptake will continue to deplete the soil solution concentration. Initially, the soil solution will be replenished by readily desorbable Co followed by less easily available Co.

After five sequential desorption periods, all the Co was desorbed from seven of the thirteen soils studied. Regression analysis was carried out using the % desorption value obtained after five sequential two hourly shakes with 0.02 M $CaCl_2$ versus the soil properties (Table 4.11). The results indica-

Table 4.9 Experiment 5(a): Adsorption and desorption characteristics of soils 1-15 after adsorption from a 14.73 μg Co ml^{-1} solution

Soil	Equilbrium concentration (μg Co ml^{-1})	Co sorbed (μg Co g^{-1} soil)	Co desorbed (μg Co g^{-1} soil)	% desorption
1	4.2	52.8	17.8	33.6
2	9.9	24.3	18.3	75.1
3	7.1	38.3	21.8	56.8
4	1.7	65.3	9.5	14.5
5	1.4	66.6	9.0	13.5
6	12.2	12.5	15.1	>100
7	9.5	26.0	17.5	67.4
8	8.0	33.5	9.4	28.1
9	2.1	63.4	13.3	21.0
10	8.2	69.5	32.0	46.0
11	8.3	69.2	30.3	43.8
12	12.3	49.0	33.8	69.0
13	5.2	84.3	23.4	27.7
14	17.4	23.4	25.4	>100
15	12.3	53.0	34.0	64.1

Table 4.10 Experiment 5(a): Regression equations relating the % desorption (y) to the various soil properties (after adsorption from 14.73 μg Co ml^{-1} solution)

Soil property	Degrees of freedom	Regression equation	SDy	Correlation coefficient (r)	t
pH	11	y = -23.7 pH + 183.3	22.13	-0.72**	
pH, % loss on ignition	10	y = -30.6 pH - 2.90% loss on ignition + 249.9	15.61	-0.87**	$t_{pH} = -6.28***$ $t_{LOI} = -3.48**$
pH, % sand	10	y = -30.3 pH + 0.98 % sand + 160.4	13.57	-0.91**	$t_{pH} = -7.31***$ $t_{sand} = 4.39*$
pH, % silt	10	y = -30.4 pH - 0.97 % silt + 243.7	17.50	-0.84*	$t_{pH} = -5.84***$ $t_{silt} = -2.76*$

Significance of correlation coefficient and t value

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

Table 4.11 Experiment 5(b): Regression equations relating the % final desorption after five sequential desorption periods (y) to the various soil properties (after adsorption from 14.73 μg Co ml^{-1} solution

Soil property	Degrees of freedom	Regression equation	SDy	Correlation coefficient (r)	t
pH	10	y = -15.67 pH + 168.6	16.96	-0.68*	
pH, % loss on ignition	9	y = -19.93 pH - 1.80% loss on ignition + 210.3	13.93	-0.80*	t_{pH} = -4.59** t_{LOI} = -2.41*
pH, % sand	9	y = -20.30 pH + 0.69% sand + 151.7	11.34	-0.87*	t_{pH} = -5.85*** t_{sand} = 3.66**
pH, % clay	9	y = -15.65 pH - 1.02 % clay + 184.0	12.87	-0.83*	t_{pH} = -4.27** t_{clay} = -2.89*
pH, % loss on ignition, % clay	8	y = -18.91 pH - 1.38% loss on ignition - 0.84% clay + 213.18	10.46	-0.89*	t_{pH} = -5.76*** t_{LOI} = -2.37* t_{clay} = -2.82*

Significance of correlation coefficient and t value

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

ted that this was strongly influenced by pH with a smaller contribution from organic matter and mineral components.

For all the soils studied, most desorption occurred during the first two desorption periods. Three groups of soils can be identified as showing different desorption characteristics (Table 4.12):

(i) Desorption complete after 5 x 2 hour sequential desorptions.

(ii) 50-99% desorption after 5 sequential desorptions.

(iii) <50% desorption after 5 sequential desorptions.

For soils in group (i) (except soil 6 which showed complete desorption in 2 hours) around 60-80% of the adsorbed Co was desorbed within the first two hours, with most of the rest desorbing by 6 hours. For soils in groups (ii) and (iii), the greatest amount of desorption occurred during the first four hours (between 50 to 75% of the total % desorption), with the rate of desorption levelling off between 4 and 10 hours to give a linear desorption rate with time. These findings can be explained in terms of the soil properties given in Table 4.1. All the soils, except soil 9 and 11, with pH <6.00 showed a rapid desorption, and belonged to group (i), while those soils in groups (ii) and (iii) had pH >6.00. Hence, pH would appear to be the main factor influencing the rate of desorption, although organic matter content was also involved, e.g. the peat (soil 9), despite a very acidic pH (pH 3.30) belonged to group (ii).

(c) Influence of time on the amount of Co desorbed

The % desorption of each soil was measured after 2, 7, 21 and 28 days (Table 4.13). For all the soils, except the two peats (soils 9 and 10) and soil 12, which had the highest % loss on ignition of the mineral soils, a decrease in % desorption was found after two days,

Table 4.12 Experiment 5(b): Sequential desorption (after adsorption from Co solution of 14.73 μg Co ml^{-1})

Soil no.	% desorption after				
	0-2 hrs	2-4 hrs	4-6 hrs	6-8 hrs	8-10 hrs
(i) Desorption complete within 10 hours					
2	78	100			
3	59	88	99	100	
6	100				
7	69	95	100		
10	50	79	97	100	
12	68	100			
15	66	98	100		
(ii) 50-99% desorption within 10 hours					
8	35	49	53	56	58
9	23	44	58	69	77
11	40	63	74	80	85
13	26	43	51	56	60
(iii) <50% desorption within 10 hours					
4	16	27	34	40	45
5	16	28	36	42	47

Table 4.13 Experiment 5(c): Influence of time on % desorption (after adsorption from 14.73 μg Co ml^{-1} solution)

Soil	% desorption after				
	0	2 days	1 week	3 weeks	4 weeks
2	75	64	56	61	56
3	57	41	40	35	36
4	15	8	8	5	7
5	14	11	8	7	8
6	100	100	100	100	100
7	67	45	39	40	40
8	28	6	7	4	5
9	21	22	24	23	24
10	46	50	52	52	56
11	41	27	28	23	23
12	66	70	72	60	63
13	24	9	8	5	6
15	63	47	45	44	44

with a further decline after one week. No major change in % desorption occurred between samples left 7 days and those left for 28 days. Soils 9, 10 and 12 showed the same % desorption irrespective of time.

For each soil, except soils 9, 10 and 12, the mean % desorption was calculated between 7 and 28 days and taken as a percentage of the quantity of Co desorbed at 0 days. The value calculated was then regressed with the soil properties (Table 4.14). Soil pH strongly influenced % desorption after 7 to 28 days with both soil texture and reducible manganese (but not exchangeable manganese) having some effect on the desorbability of Co with time. From Table 4.14 it can be seen that as reducible Mn content increases, desorption declines.

(d) Discussion of the factors determining % Co desorption

Soil acidity is the main factor determining the proportion of adsorbed Co which remains in a form which is readily desorbed. Very little work has been published on the desorption characteristics of Co but information is available on the efficacy of Co application to pasture as a means of counteracting Co deficiency. In such circumstances soils of naturally high pH or limed soils have been observed to have very low residual values (Nicolls and Honeysett, 1964). The work described here suggests this may be a direct effect of strong Co binding to adsorption sites at high pH. In addition, for mineral soils, a variable amount of adsorbed Co, although initially readily desorbable, becomes more tightly bound with time. In organic soils, a greater proportion remains desorbable compared to mineral soils, irrespective of how long the sample is left. This may explain the fact that when Co is applied to peats in the field the effect of $CoSO_4$ treatment remains for as long as

Table 4.14 Experiment 5(c): Regression equations relating mean % desorption between one and four weeks expressed as a fraction of the initial % desorption (y) against various soil properties (after adsorption from 14.73 μg Co ml^{-1})

Soil property	Degrees of freedom	Regression equation	SDy	Correlation coefficient (r)	t
pH	10	$y = -23.3\ pH + 192$	10.67	-0.92***	
pH, manganese (reducible)	9	$y = -24.59\ pH - 0.12\ Mn_{red} + 209$	8.55	-0.95**	$t_{pH} = -9.88***$ $tMn_{red} = -2.56*$
pH, % sand manganese (reducible)	8	$y = -27.33\ pH + 0.37\%\ sand - 0.14\ Mn_{red} + 203$	4.80	-0.98**	$t_{pH} = -17.97***$ $t_{sand} = 4.54**$ $tMn_{red} = -5.36***$
pH, % silt manganese (reducible)	8	$y = -28.08\ pH - 0.44\ \%\ silt - 0.17\ Mn_{red} + 242$	5.77	-0.98**	$t_{pH} = -14.31***$ $t_{silt} = -3.43**$ $tMn_{red} = -4.84**$

Significance of correlation coefficient and t value

*P=0.05; **P=0.01; ***P=0.001

thirteen years while some mineral soils show a poor residual effect of $CoSO_4$ sometimes in as short a time as two years (Evans, 1985; Klessa et al, 1988).

As solution Co concentrations decline all the soils studied showed that some of the adsorbed Co can be released to replenish the soil solution. For the majority of the soils investigated almost all the adsorbed Co was released after incubating five times with a Co-free solution. In soil, such abrupt changes in soil solution concentrations will not occur. Instead, plant uptake will slowly reduce solution Co leading to a gradual depletion rather than a dramatic change in Co concentration. Thus, in the field situation a proportion of the Co measured as readily desorbable (i.e. after 2 hours incubation) is likely to become fixed as a result of the longer time the Co remains on the adsorption site. The situation is further complicated by various plant factors. Plant roots release various exudates which alter the soil conditions in the vicinity of the root surface. For example, some of the organic exudates produced may have a strong affinity for Co and desorb Co which is tightly bound and which might not be desorbed by salt extraction. Therefore, although the results from the sequential desorption experiment indicate what might happen as plant uptake reduces soil Co concentration in solution other factors must also be considered.

In the present study, both organic matter and soil texture were identified as the most important factors determining Co desorption in the short term with texture and reducible Mn content being more important over longer periods. When individual soil components were investigated, McLaren et al (1986) found that Co adsorbed by humic acid and montmorillonite was much more readily desorbed than

that held by soil oxides. Further, he suggests that with time Co will become redistributed and be found mainly in association with the stronger binding soil oxides. In this respect, McKenzie (1967) proposed that the mechanism of Co adsorption on to Mn oxide minerals involved a slow irreversible migration into the actual oxide lattice. Initially, Co remaining on the surface will be relatively easily desorbed, the ease of desorption declining with time. In addition, some of the desorption characteristics attributed to the clay and silt fractions will actually be due to soil oxides or organic material coating mineral particles. Hydrous oxides found in association with silt and clay particles will adsorb Co more strongly than the clay particles themselves (McLaren et al, 1986) and so reduce the amount desorbed from clay and silt.

4.3.4 Experiment 6 - Influence of pH on Co adsorption and desorption

As expected, pH has a large effect on the quantity of Co adsorbed and desorbed. With increased pH, the amount of Co adsorbed increased (Table 4.15). Almost total adsorption of the added Co occurred at pH >7.0 and over the range pH 4.0-7.0 the amount of Co adsorbed increased linearly with increasing pH. Desorption became more difficult at high pH values (Table 4.16). At a pH <5, all the adsorbed Co was released during the first desorption period. As pH increased the amount of Co desorbed during the first period was reduced progressively. At pH >6, less than half of the adsorbed Co was released even after five sequential desorptions with the two soils of highest pH (around pH 8).showing no desorption of Co. Hence, in practical terms, application of Co in the field to similar soils of pH >6.0 will probably be of little benefit in elevating herbage Co concentrations. At such pH

Table 4.15 Experiment 6: Influence of pH on adsorption from an initial Co solution of 14.73 μg Co ml^{-1}

pH	Equilibrium concentration (μg Co ml^{-1})	Co sorbed (μg Co g^{-1} soil)	% of maximum adsorption
4.0	13.3	7.1	9.6
4.9	12.2	12.5	16.9
5.3	8.4	31.8	43.2
5.7	5.8	45.0	61.6
6.0	3.4	56.7	76.9
7.0	0.8	69.4	94.3
7.5	0.3	72.3	98.2
8.0	0	73.7	100
8.2	0	73.7	100

Table 4.16 Experiment 6: Influence of pH on desorption after an initial adsorption from a Co solution of 14.73 μg Co ml^{-1}

pH	% desorption after				
	0-2 hrs	2-4 hrs	4-6 hrs	6-8 hrs	8-10 hrs
4.0	100				
4.9	100				
5.3	56.7	78.1	84.5	91.8	92.0
5.7	ND	50.4	52.5	53.0	53.0
6.0	25.9	41.2	48.9	49.0	49.0
7.0	7.7	14.2	18.1	21.6	24.7
7.5	2.7	5.4	7.1	8.6	10.0
8.0	0	0	0	0	0
8.2	0	0	0	0	0

ND = not determined

values the irreversible binding of Co to the adsorption sites, reduces the availability of the applied Co.

The role of pH in determining Co adsorption and desorption has been investigated by various workers (Healey et al, 1968; Murray, 1974, 1975; Loganathan et al, 1977). Healey et al (1968) found almost complete adsorption of added Co at pH 7 with an abrupt increase in Co adsorption between pH 6.5 and 7.5.

At low pH, Co will be held by cation exchange on the surfaces of organic materials, clays and manganese oxides.

For pH >3 (point of zero charge of carboxyl groups present on organic matter) all these surfaces will carry a negative charge, i.e. the organic matter from the hydrolysis of carboxyl groups, clay minerals from the permanent negative charge induced during clay synthesis and manganese oxide minerals from the hydrolysis of surface hydroxyls (point of zero charge pH 1-5 (Murray, 1974)). As pH increases further additional hydrolysis of hydroxyl groups of the Mn mineral surfaces and clay edges will produce more negatively charged sites which can attract and bind Co. At low pH, specific bonding of Co is fairly weak and desorption appears to occur readily.

At high pH (pH 6.0-8.0) adsorption increases. This can be accounted for by various reasons:

(i) *Increased negative charge* due to the dissociation of surface hydroxyl groups present on hydrous oxides, e.g. point of zero charge for αAl_2O_3 is pH 7.6 and for αFe_2O_3 is pH 8.5 (Murray, 1968).

(ii) *Presence of different forms of aqueous Co.* Loganathan et al (1977) suggest that at pH 6.0 the concentration of the first hydrolysis product, $CoOH^+$, increases to significant amounts and may show preferential adsorption compared to Co^{2+}, i.e. $Co^{2+} + H_2O \rightleftharpoons CoOH^+ + H^+ \quad pK_1 = 4.4$

However, Healey et al (1968) suggest that Co^{2+} remains the dominant species in solution at pH 6.5 to 7.5 and concluded that the hydroxy form could not account for increased adsorption.

(iii) *Precipitation of cobalt hydroxide.* At pH 9.0 (Jenkins and Jones, 1980), precipitation of cobalt hydroxide ($Co(OH)_2$) occurs. Loganathan et al (1977) propose that interface "precipitation" of cobalt hydroxide is seen even when conditions are not achieved for the formation of $Co(OH)_2$ in the bulk solution. Further, Healey et al (1968) proposed that the hydroxide concentration immediately next to the adsorbing surface may be higher than that found in soil solution and nucleation of a hydroxide precipitate may occur at pH values lower than would be expected in the bulk solution. Hence, the increased adsorption at higher pH may be due to precipitation of $Co(OH)_2$ on the various adsorbent surfaces. Such forms will be unavailable for plant uptake and no Co will be desorbed when placed in a Co-free solution.

To conclude, Co sorbed at low pH is bound by relatively weak bonds and is readily desorbed, but as pH increases the bonding involved becomes stronger and at high pH precipitation as cobalt hydroxide may prevent desorption from taking place.

4.3.5 Experiment 7 - Prediction of Co adsorption/desorption characteristics of soil

The objective of this work was to study the adsorption and desorption properties of soil in relation to the use of cobalt sulphate and its residual value. In order to carry this out a range of soils was required for which the effectiveness of Co treatment of pasture was known. As detailed in section 4.2 only two such soils were obtained and this section of the work could not be carried out. However, these soils did provide the opportunity to compare the measured and predicted values of adsorption and desorption of Co using the following relationships:

(i) Amount of Co adsorbed from an initial solution of 7.37 μg Co ml^{-1}.

(ii) Gradient of the adsorption isotherm.

(iii) % desorption after adsorption had taken place from a solution containing 14.73 μg Co ml^{-1}.

The properties of the two soils are given in Table 4.1. Adsorption and desorption were measured by the methods used previously and compared with the values obtained after substitution of the soil properties into the various equations (Table 4.17). In order to assess the ability of the various regression equations to predict the adsorption/desorption properties of soils the differences between the measured and predicted values were determined:

(i) Amount of Co adsorbed from an initial solution at 7.37 μg Co ml^{-1}

All the equations gave values which were in reasonable agreement with the measured values.

Table 4.17 Experiment 7: Comparison between actual and calculated values for the various regression equations

Properties	Predicted value for soils		Measured value for soils	
	17	18	17	18
(i) Amount of Co ($\mu g\ g^{-1}$) adsorbed from a 7.37 μg Co ml^{-1} solution				
pH, % loss on ignition	31.4	32.5		
*pH, % sand	41.1	35.7	35.7	34.1
pH, % silt	30.4	37.1		
(ii) Gradient of adsorption isotherm				
pH, % loss on ignition	25.6	27.3		
pH, % sand	41.3	31.5	65.8	32.6
*pH, % loss on ignition; % clay	42.1	22.6		
(iii) % desorption after incubation with 14.73 μg Co ml^{-1} solution				
pH	45.5	32.6		
pH, % loss on ignition	24.8	17.3	0.72	5.1
*pH, % sand	2.8	7.9		
pH, % silt	26.4	2.6		

*equations giving the highest correlation coefficients

(ii) The gradient of the adsorption isotherm

In general, the equations tended to under-estimate the gradient of the isotherm. This was particularly so for soil 17, where all the equations gave a predicted value of almost half that of the measured value. Soil 18 gave better agreement with the equation involving pH and % sand giving very similar values to those obtained experimentally.

(iii) % desorption after adsorption from 14.73 μg Co ml^{-1} solution

The equations over-estimated the values with soil 17 giving the poorest agreement.

Hence, the regression equations formed to predict various adsorption and desorption properties of soil gave reasonable agreement with values obtained experimentally and show promise as a method of predicting the Co adsorbing and desorbing characteristics of unknown soils. The prediction of desorption appears to be the more difficult. A lot more work is required in order to form a more accurate model such as a study involving more soils with a wider range of soil properties. However, this work highlights the areas requiring investigation. For example, soil 17, which produced the poorest agreement with the various models, contained almost double the reducible Mn content of any of the soils used in formulating the various equations. At such high levels, reducible Mn may be more important than was previously suggested. In addition, other properties, in particular the role of hydrous oxides merits further experimentation before a model to predict the residual value to Co application can be produced.

Despite soils 17 and 18 showing almost identical adsorption from 7.37 μg Co ml^{-1} solution, large differences were observed in

their residual response to cobalt sulphate application (the application of 0.6 kg Co ha^{-1} as hydrated cobalt sulphate remained effective for less than one year for soil 17, compared with over three years for soil 18). Therefore, a single adsorption value cannot be used to determine the likely response to cobalt sulphate application in the field. On the other hand, both the measured gradient of the Q/I curve and the % desorption after an incubation with a solution containing 14.73 µg Co ml^{-1} did differentiate between the two soils in that soil 17 which had the greater buffering capacity for Co from adsorption studies showed the poorer residual effect of applied cobalt sulphate. These indices would appear to show more promise as predictive equations but as stated earlier require further study in order to formulate a more accurate model.

CHAPTER 5 - THE INFLUENCE OF pH AND GRASS SPECIES ON HERBAGE COBALT

5.1 INTRODUCTION

In recent times land improvement schemes have been identified as producing a greater incidence of Co deficiency in grazing ruminants (COSAC/SARI, 1982). Such systems involve liming and the introduction of better quality grasses which have both been recognised as major factors reducing herbage Co content.

Various workers have shown that herbage Co concentrations decrease with increased pH and are particularly affected by liming (Ekman, 1952; Hill et al, 1953; Wright and Lawton, 1954; Percival et al, 1955; Archer, 1955; Mitchell, 1972; McLaren et al, 1987; Klessa et al, 1988). However, for soils with pH ≥ 6, further liming appears to have little effect (Wright and Lawton, 1954; McLaren et al, 1987; Klessa et al, 1988). For soils with pH <6, small changes in soil acidity result in large alterations in herbage Co concentrations.

The differing Co concentrations of various grass species grown under the same conditions have been well documented (Hill et al, 1953; Mitchell, 1954; Mitchell et al, 1957; Voss and MacPherson, 1977; Klessa et al, 1988), but the Co content of grass species normally used in an upland re-seed has not been examined specifically.

Two experiments were set up to investigate both the role of pH and grass species on herbage Co. In a glasshouse pot trial, experiment 1, the influence of pH on grasses recommended for an upland reseeded pasture was studied. By using a soil known to produce Co-deficient pasture, the effect of pH was investigated on

an unlimed and limed soil (pH 5.0-6.5). The aim of this work was to identify the critical pH values, above which pasture Co concentrations fall below the deficiency threshold, as well as determining the grass types of most importance in governing the Co content of the sward. In addition, the work aimed to see whether all grass species are equally affected by pH or whether each grass type shows a different response to changes in pH. In contrast Experiment 2, examined the effect of species on the Co content of swards in the field. At two different locations, the same grass types were grown under a low or borderline soil Co status, in order to determine whether such soils produced similar trends in Co concentrations for the various grasses.

The aims of these two experiments were as follows:

(i) To quantify the relative effect of soil acidity and the herbage Co content of various grass species.

(ii) To investigate the critical pH values above which Co status of the pasture is adversely affected.

(iii) To study how various grass seed mixtures will affect Co content of the sward and, thus, influence the supply of Co to the grazing ruminant.

5.2 MATERIALS AND METHODS

5.2.1 Soils

The location of the soil (soil 1) used in the pot trial (Experiment 1) is given in Table 5.1. Such a soil is regarded as being highly likely to produce Co-deficient pasture (COSAC/SARI, 1982). A representative sample was obtained of the topsoil (0-10 cm) and was then riddled, dried and milled to <2 mm as detailed in Chapter 3.

Table 5.1 Location and properties of soils used in the experiments to determine the influence of grass species and pH on herbage Co concentrations

(a) Location and soil type

Soil no.	Experiment ref.	Location	Grid reference	Soil association	Soil series	Drainage status
1	1	Glasshouse Investigational Unit, W.S.C.	NS383232	Dreghorn	Dreghorn	Free
2	2	Balig Farm, Ayr	NS307173	Darleith	Darleith	Free
3	3	Sclenteuch, Straiton	NS396073	Peat	Peat	Peat

(b) Soil properties

Soil no.	pH	% loss on ignition	% sand	% silt	% clay	Available Co mg kg^{-1}	Exchangeable Mn mg kg^{-1}	Reducible Mn mg l^{-1}	Available P mg l^{-1}	Available K mg l^{-1}	Extractable Mg mg l^{-1}
1	5.4	5.1 (L)	77.8	11.3	10.9	0.45 (L)	6.6 (M)	153	20 (L)	34.9 (VL)	41 (L)
2	5.6	27.1 (M)	35.2	30.3	34.4	0.70 (M)	20.9 (M)	64.5	-	-	-
3	6.5	82.6 (VH)	-	-	-	0.24 (L)	19.9 (M)	16	-	-	-

VL = very low; M = moderate; L = low; VH = very low

Details of the location of the two field sites (soils 2 and 3) used in Experiment 2 are given in Table 5.1.

The properties of the soils (Table 5.1) were determined on soil samples taken at the beginning of each experiment by the methods given in Chapter 3.

5.2.2 Soil pH adjustment (Experiment 1)

For soil 1, the amount of calcium hydroxide required to obtain a range of pH values was determined by carrying out a lime requirement determination as detailed in Chapter 3. The nominal pH levels chosen for the study were as follows:

Individual grass species: pH 5, 5.5, 6 and 6.5;
Grass mixture: pH 5, 5.5, 5.75, 6, 6.25 and 6.5.

5.2.3 Grass species

(a) Experiment 1 - Grass species and pH pot trial

"Lambhill/86" mixture (Sinclair McGill, Scotland plc) was chosen as the study material. This mixture is recommended for use in an upland re-seed (Watson, 1985). As well as using the mixture all the individual constituent grasses (made up of the cultivars used in the correct ratio) were investigated. The "lambhill/86" mixture was composed of (cultivar in brackets):

62% Perennial ryegrass (3 Melta Tetra:2 Premo:2.5 Morenne: 1 Preference:2 Springfield)
9% Italian ryegrass (Dalita)
14% Timothy (2 Goliath:1 Kampe)
6% Red fescue (Echo)
3% Meadow grass (Erite smooth stalked)
6% White clover (8 Huia:1 Kent wild)

(b) Experiment 2 - Grass species field trial

Grass samples were obtained from two sites (Table 5.1) established by the "Agronomy Department, West of Scotland College". These were set up as "Observation Plots" to study grass species used or suggested for use in upland re-seed mixtures, as affected by ease of establishment, frost hardiness and durability. The grasses were sown on 25th August 1983 at Balig Farm (soil 2) and 12th August 1986 at Sclenteuch Farm (soil 3) in single 1.2 m x 4 m plots with a small discard of around 20 cm between plots. The grass species with their respective cultivars are given in Table 5.2. As no clover plots were available at Balig, clover samples were obtained from the discards after separation from the grasses, while at Sclenteuch clover was taken from grass/clover plots. Hence, at both sites an indeterminate mixture of both white and red clover was obtained made up of various cultivars. For the grass species made up of different cultivars, each cultivar was sampled from its respective plot and bulked with the other cultivars to give one composite sample.

5.2.4 Establishment of grass and pH pot trial

For each pot, 2 kg of dried, milled soil (<2 mm) was mixed by hand in a bucket with 2 litres of perlite. Dried, milled soil, although producing a homogenous mixture, lacks soil structure and the percolation of water through the soil tends to be slow, increasing the risk of waterlogging. As Co availability is strongly influenced by drainage status, inert perlite was added to increase the rate of water movement. Each pot received a basal dressing equivalent to 60 kg N ha^{-1}, 130 kg P_2O_5 ha^{-1},

Table 5.2 Grass species investigated at Balig and Sclenteuch

SPECIES (with cultivars in brackets)	
Balig	Sclenteuch
Clover (various cultivars of both white and red clover)	Clover (various cultivars of both white and red clover)
Italian ryegrass (Optima)	Italian ryegrass (Optima)
Perennial ryegrass (S23, Peruna, Melle, Parcour, Meltra, Barclay, Barry, Bellatrix, Fiesta, Hunter, Player, Royal)	Perennial ryegrass (Barclay, Bellatrix, Stormont Zephyr, Elka, Contender, Peruna, Coudesa, Meltra, Springfield)
Cocksfoot (Cambria)	Cocksfoot (Cambria)
Crested dog's tail (Southlands)	Crested dog's tail (Southlands)
Timothy (Goliath)	Timothy (Barvanti)
Yorkshire fog	Yorkshire fog
Meadow foxtail	-
Sweet vernal	-
-	Poa compressor (Reubens)
Bent (Emarold)	Bent - Velvet (Kingstrom, creeping (Penn Cross), Highland
Fescue-red (Cascade, Jamestrown)	Fescue - Chewing (Center)
Vetch (Winter)	-
Meadow grass (Baron)	Rough stalked meadow grass

100 kg K_2O ha^{-1} as ammonium nitrate, calcium hydrogen phosphate and potassium chloride respectively, calculated to supply the plants' requirement of N, P, and K (SAC, 1985) plus the appropriate amount of calcium hydroxide to obtain the desired pH (see 5.2.2). After thorough mixing the soils were transferred to plastic pots containing four draining holes and slits around the sides to ensure adequate drainage. Before sowing, each pot was wetted up with tap water and the seeds then scattered over the surface with a 1 cm layer of perlite placed over the top. The seeding rate was 2.5 g pot^{-1} for the mixture, perennial ryegrass, Italian ryegrass, timothy, red fescue and meadow grass, and a higher rate of 5 g pot^{-1} for white clover. These rates were chosen in order to obtain a high plant density.

The pots were arranged in a randomised block design of four blocks on four trestle tables in the glasshouse. Each block contained 30 treatments (Table 5.3) arranged in a randomised 6 x 5 arrangement. No artificial lighting or heating was used since it was difficult to ensure constant conditions throughout the glasshouse. The pots were watered using tap water in a plastic watering can to the draining tray and soil surface as required.

Six weeks after sowing, all the grasses showed poor, patchy growth. On examination of the surface of the pots it was found that the surface perlite had formed a compacted layer through which it was difficult for the grasses to penetrate. Due to disturbance in the transference of the pots from the laboratory to the glasshouse both the seeds and surface perlite had become redistributed to give patches containing a large number of seeds below a thick 3 cm layer of perlite. To rectify this problem all

Table 5.3 Experiment 1: Experimental treatments for grass species and pH pot trial

Grass	Nominal pH					
	5	5.5	5.75	6	6.25	6.5
Mixture	√	√	√	√	√	√
Perennial ryegrass	√	√		√		√
Italian ryegrass	√	√		√		√
Timothy	√	√		√		√
Red fescue	√	√		√		√
Meadow grass	√	√		√		√
White clover	√	√		√		√

the surface perlite and grass was removed from each pot. The seeds were re-sown at the same rates as before, except for the white clover which was reduced to 2.5 g pot^{-1} since the previous rate appeared to be too high. A thin layer of very fine grade perlite was placed over the seeds and water supplied as a fine mist to the soil surface in an attempt to reduce the danger of surface capping, with the bulk of the water put in the draining tray.

While these measures were successful for most of the grasses, the finer grasses of red fescue and meadow grass continued to show poor growth six weeks later. For these grasses, the plants were removed and re-sown. Before re-sowing it was noted that the soil had become very compacted as a result of surface watering and this had reduced root penetration. Therefore, before re-sowing the compacted soil was broken up. The stronger roots of the other grasses appeared to be able to pass through the barrier and their growth was not inhibited.

Approximately one month after germination, the pots containing the mixture, timothy, perennial ryegrass and Italian ryegrass showed signs of nitrogen deficiency. Therefore, a liquid feed was used containing 3 N:1 P_2O_5:1 K_2O as monoammonium phosphate, potassium nitrate and calcium nitrate respectively. The amount of feed given was adjusted according to the physical appearance of the plant. In addition, a magnesium feed was given to the clover pots as 150 g^{-1} magnesium sulphate.

5.2.5 Sampling and analysis

(a) Experiment 1 - Grass species and pH pot trial

(i) Soil samples were taken from each pot at the start and finish of the experiment by the method described in Chapter 3. Only pH was determined on air-dried soil.

(ii) Herbage samples were taken on two dates when grass height was around 15-20 cm, namely 20th June 1986 and thirty-one days later (21st August 1986) by the method described in Chapter 3. The first cut corresponded to 118 days after sowing for the mixture, clover, perennial ryegrass, Italian ryegrass and timothy, and eighty-two days after sowing for red fescue and meadow grass. Yields for all the pots were measured and the herbage analysed for Co, Fe, Mn, Zn and Mo as described in Chapter 3.

(b) Experiment 2 - Grass species field trial

Two cuts were taken at each site as follows:
Balig Farm (soil 2) - 28/5/87 and 7/9/87.
Sclenteuch (soil 3) - 11/6/87 and 16/9/87.

Representative samples were taken from each plot using sheep shears, cutting at about 4 cm above the soil surface. The plots were then trimmed using an "Agria" mower and the grass discarded. Plant yields were not monitored. All herbage samples were analysed for Co and Fe as detailed in Chapter 3.

5.3 RESULTS

5.3.1 Experiment 1 - Grass species and pH pot trial

The mean pH values for each treatment at the start and finish of the trial are given in Table 5.4 (a) and (b) respectively. Initially all the pH values were around 0.5 pH unit greater than the nominal pH, probably because the lime requirement had been determined on only the soil rather than the soil/perlite mixture. Although the initial pH of the pots for the various species was not significantly different, the various grasses produced significant effects on the final pH. Perennial ryegrass, timothy, meadow grass and red fescue all showed an increase in soil pH over the experimental period, while the mixture and Italian ryegrass pots showed little change in soil pH over the same time, compared with a dramatic drop in pH (approximately 0.6 of a pH unit) seen for the white clover pots.

While dry weight yields varied between the different species, this was mainly due to the varying lengths of time that the plants had been growing than to a direct species effect on yield. Hence, this aspect will not be discussed further.

The mean herbage Co concentrations of the various treatments are given in Tables 5.5 (a) and (b). All the values were very high, with even the lowest concentration well in excess of the minimum requirement of grazing ruminants ($\geq$0.10 mg Co kg^{-1} DM). This effect was even greater in the second cut. In some cases the expected trends in herbage Co as influenced by pH were seen, e.g. first and second cut Italian ryegrass showed a decline in Co concentrations with increased pH, while both cuts of meadow grass produced higher Co concen-

Table 5.4 Experiment 1: Influence of grass species and nominal pH on measured pH

(a) Initial pH (n = 4)

		Grass species							
		Mixture	Perennial ryegrass	Italian ryegrass	Timothy	Meadow grass	Red fescue	White clover	Mean
Nominal pH	5.0	5.4	5.4	5.5	5.4	5.4	5.5	5.4	5.4
	5.5	5.7	6.2	6.0	5.9	6.0	5.9	6.1	6.0
	6.0	6.7	6.3	6.6	6.7	6.6	6.7	6.5	6.6
	6.5	7.1	7.1	7.2	7.3	7.3	7.3	7.4	7.2
Mean		6.2	6.2	6.3	6.3	6.3	6.4	6.3	

S.E. = 0.091

(b) Final pH (n = 4)

		Grass species							
		Mixture	Perennial ryegrass	Italian ryegrass	Timothy	Meadow grass	Red fescue	White clover	Mean
Nominal pH	5.0	5.7	5.9	5.7	6.1	6.1	6.2	5.0	5.8
	5.5	5.7	6.1	5.9	6.2	6.5	6.3	5.5	6.0
	6.0	6.4	6.5	6.3	6.7	7.1	7.1	5.9	6.6
	6.5	7.0	7.3	7.0	7.4	7.6	7.3	6.4	7.1
Mean		6.2	6.4	6.2	6.6	6.8	6.7	5.7	

S.E. = 0.123

Table 5.5 Experiment 1: Influence of grass species and pH on herbage Co concentration (mg Co kg^{-1} DM)

(a) First cut (n = 4)

		Grass species							
		Mixture	Perennial ryegrass	Italian ryegrass	Timothy	Meadow grass	Red fescue	White clover	Mean
Nominal pH	5.0	0.59	0.52	0.51	0.42	0.25	0.24	0.80	0.48
	5.5	0.62	0.54	0.49	0.37	0.18	0.24	0.37	0.40
	6.0	0.53	0.40	0.39	0.20	0.27	0.18	0.36	0.33
	6.5	0.41	0.56	0.34	0.20	0.40	0.15	0.54	0.37
Mean		0.53	0.51	0.43	0.30	0.28	0.20	0.52	

S.E. = 0.051

(b) Second cut (n = 4)

		Grass species							
		Mixture	Perennial ryegrass	Italian ryegrass	Timothy	Meadow grass	Red fescue	White clover	Mean
Nominal pH	5.0	0.68	0.65	0.58	0.31	0.37	0.37	1.86	0.69
	5.5	0.81	0.89	0.53	0.33	0.32	0.46	0.95	0.61
	6.0	0.83	0.69	0.40	0.25	0.53	0.46	0.77	0.56
	6.5	0.78	0.96	0.33	0.30	0.60	0.43	0.88	0.61
Mean		0.78	0.80	0.46	0.30	0.45	0.43	1.12	

S.E. = 0.0806

trations as pH increased. Generally, herbage Co concentrations were greatest in the white clover, with the lowest values obtained with timothy. However, this was not always the case.

5.3.2 Experiment 2 - Grass species field trial

The Co concentrations of the different grasses for the two cuts taken at the two sites are given in Table 5.9. At Balig (soil 2), herbage Co concentrations ranged from 0.04-0.15 mg Co kg^{-1} DM for the first cut and from <0.02-0.32 mg Co kg^{-1} DM for the second cut. In neither cut did clover contain the highest Co contents. The second cut samples gave a mean Co concentration below the minimum requirement of grazing ruminants (≥0.10 mg Co kg^{-1} DM) with the first cut classed as producing pasture of borderline Co contents. In general, the herbage Co concentrations at Sclenteuch were below those obtained at Balig and for both cuts, all samples (except the first cut clover) were below the minimum requirement for ruminants. For both cuts, clover produced the highest Co contents.

5.4 DISCUSSION

5.4.1 Experiment 1 - Grass species and pH pot trial

(a) The high herbage Co concentrations

All the herbage samples contained very high Co contents, well in excess of those obtained by McLaren et al (1987) in a pot trial involving sixteen different soils. The soil used gave an extractable Co value which was classed as low (Table 5.1) and regarded as highly likely to produce Co-deficient herbage. In a pot, plant roots

can explore the whole soil volume since the physical barriers to root penetration are normally less than those which occur naturally in the field. Hence, higher Co contents could be expected in pot experiments compared with field trials. However, this alone cannot account for the excessively high Co contents. Unexpectedly high herbage Co concentrations can often be accounted for in terms of soil contamination (Fleming, 1985) with the presence of even small amounts of soil adhering to the herbage resulting in elevated Co contents. In this study, Fe concentration in herbage was taken as a measure of contamination by soil. Values of >200 mg Fe kg^{-1} DM are normally regarded as indicative of the presence of enough soil to have a significant influence on measured herbage Co contents (Dixon, 1987). For this trial the mean values (n = 120) obtained at each cut were:

First cut 148 ± 6.5 mg Fe kg^{-1} DM (± SE)

Second cut 93 ± 4.3 mg Fe kg^{-1} DM

Therefore, soil contamination was low and any values of >200 mg Fe kg^{-1} DM could be explained in terms of a treatment effect, for example, low pH, plant species.

After examining various possibilities, the reason for the high herbage Co contents was finally tied down to the anomalously high Co concentrations in the perlite. Perlite is an inert alumino-silicate of volcanic origin, with virtually no cation exchange capacity, composed of 73% silicon dioxide and 13% aluminium oxide and is regarded as containing negligible amounts of plant nutrients (Bunt, 1976). However, the batch of perlite used here contained an acetic acid extractable Co content of 1.74 mg Co kg^{-1} (other batches were found to contain

between <0.01 and 0.11 mg Co kg^{-1}) and when mixed with soil produced a growth medium containing approximately 1.16 mg Co kg^{-1} (as acetic acid extractable). Therefore, instead of a low Co growth medium, the plants were grown under Co abundant conditions. Thus, the emphasis of the experiment was changed from an investigation of Co uptake from a low Co soil to one containing large quantities of Co. As each pot contained equal amounts of perlite it was assumed that each treatment was grown under similar high soil Co conditions.

(b) Influence of grass species

The mean Co concentrations for each cut showed that clover contained the most Co, with timothy, meadow grass and red fescue the lowest (Table 5.5). However, this was not always the case at each nominal pH value. Although many workers have noted a relatively high Co content in clovers (Mitchell et al, 1957; Adams et al, 1969; Reith et al, 1983; Klessa et al, 1988), others have found that under some conditions grasses may contain similar or more Co than clovers (Mitchell, 1960; Andrews, 1966; McLaren et al, 1987). Further, Mitchell (1960) suggested that under low soil Co conditions little difference in Co contents is seen between clover and grasses as opposed to substantial variations when soil Co status is adequate. In this experiment, however, even with very high soil Co concentrations, differences in the Co contents of clover and grass were very variable. During the course of the trial plant growth was inhibited both by the impediment of root growth and by nutrient deficiencies (see section 5.2.4). However, not all the replicates of treatments were equally affected, since some maintained good growth rates while others were inhibited.

Under natural conditions, maturation rates vary between different species with a consequent influence on Co content (Fleming, 1970). In this experiment, this effect was confounded by the fact that for each species the rate of maturation was highly variable depending on the extent to which growth had been reduced. For example, for some of the white clover, magnesium deficiency slowed down growth and maturation while in other pots, unaffected by the deficiency, the white clover continued to grow vigorously and reached maturity more quickly. Hence, interpretation of the data is difficult and only broad generalisations can be made. These suggest that clover contained higher Co contents than the grasses.

Although initially no significant differences in soil pH were seen between the different species, the final pH was significantly influenced by plant species (Table 5.4). For example, white clover showed a strong acidifying effect, with a drop in pH of around 0.5-0.9 pH unit from initial values. It has been recognised that N_2-fixation in root nodules of legumes can result in a reduction in rhizosphere pH (Salisbury and Ross, 1978), but normally no effect is seen in bulk pH. In this present experiment where root growth was extensive most of the soil was in contact with roots and hence, bulk pH was affected. By contrast, timothy, meadow grass and red fescue all appeared to cause an increase in bulk soil pH. No-one has attempted to explain the variable Co content of different species in terms of these changes in pH. The relatively high Co content of clover may result partly from rhizosphere acidification enhancing Co availability, with the alkalising influence of timothy and other grasses having a detrimental effect on the availability of Co.

(c) Influence of pH

The effect of pH on herbage Co contents for some species did show the expected trends, while for others it had no influence or produced the opposite effect. Various workers have demonstrated a decline in herbage content with increased pH (McLaren et al, 1987; Klessa et al, 1988). For the first cut, the mixture, white clover, Italian ryegrass, timothy and red fescue all produced negative correlation coefficients when herbage Co content was regressed against pH (Table 5.6). By the second cut, however, only the white clover, Italian ryegrass and meadow grass showed this trend. All other species produced opposite trends, although these were not significant except for first cut meadow grass.

A possible explanation for these conflicting results may lie in the fact that the pH differences between pots was too small to produce any effect on trace element uptake. Both Zn and Mo concentrations in herbage are strongly influenced by pH (Mitchell, 1964) with Zn availability reduced and Mo uptake increased at high pH. In a regression analysis, involving mean soil pH and herbage Zn and Mo concentrations, the expected negative and positive correlation coefficients, respectively, were obtained (Table 5.7). Hence, the differences in pH did influence availability of trace elements, and therefore, final plant concentrations. This suggests that although pH was potentially able to influence Co availability, other factors masked this effect.

During the course of this experiment, many of the pots became waterlogged. The soil/perlite mixture tended to slump to

Table 5.6 Experiment 1: Regression analysis of herbage Co concentration (y) versus soil pH

Species	First cut		Second cut	
	Regression equation	r	Regression equation	r
Mixture	y = 1.39 - 0.14 x	-0.54**	y = 0.54 + 0.05x	0.17
White clover	y = 1.28 - 0.13x	-0.29	y = 4.16 - 0.51x	-0.62*
Italian ryegrass	y = 1.20 - 0.12x	-0.49*	y = 1.54 - 0.17x	-0.58*
Perennial ryegrass	y = 0.46 + 0.01x	0.27	y = 0.15x - 0.18	0.34
Timothy	y = 1.19 - 0.14x	-0.82***	y = 0.39 - 0.01x	-0.23
Meadow grass	y = 0.10x - 0.35	0.50*	y = 0.17x - 0.68	0.67**
Red fescue	y = 0.77 - 0.08x	-0.50*	y = 0.18 + 0.04x	0.18

Level of significance of correlation coefficient (r)

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

Table 5.7 Experiment 1: Correlation coefficients obtained from regression analysis of herbage Zn and Mo concentrations versus soil pH

Species	First cut		Second cut	
	Zn r	Mo r	Zn r	Mo r
Mixture	-0.80***	0.84***	-0.82***	0.80***
White clover	-0.63**	0.89***	-0.33	0.84***
Italian ryegrass	-0.72***	0.62**	-0.92***	0.54*
Perennial ryegrass	-0.69**	0.74***	-0.75***	0.78***
Timothy	-0.56*	0.33	-0.74***	0.29
Meadow grass	-0.21	0.21	-0.48	0.21
Red fescue	-0.26	0.78***	-0.46	0.72**

Level of significance of correlation coefficient

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

produce a compacted mass through which water passed only very slowly. Co availability has been shown to be strongly influenced by drainage status (Mitchell, 1960, 1964, 1972; Berrow, 1985), with herbage Co concentrations increasing under poorly drained conditions. Whereas the availability of Zn and Mo are not affected by redox potential, the concentration of Mn in soil solution increases with waterlogging as a result of the breakdown of Mn containing minerals with the consequent release of Co held within the Mn mineral lattice (Wild, 1988). Despite soil acidity having a strong influence on herbage Mn contents (Wild, 1988), no significant correlation coefficients were obtained for herbage Mn concentrations versus pH. Thus, it would seem that the observed trends in Mn concentrations were probably due to waterlogging which confounded the pH effect lending further evidence that Co availability was similarly affected.

In order to obtain an indication of which factors were influencing Co availability a regression analysis of herbage Co against herbage Mn concentrations was performed (Table 5.8). For second cut herbages, significant positive correlation coefficients were obtained for all species except meadow grass and red fescue with the latter being almost significant. From Table 5.6 it can be seen that meadow grass was the herbage species which produced the most significant relationship between herbage Co and soil pH at the second cut. For the first cut material the relationship between Mn and Co herbage concentrations was less marked. The Co concentration of first cut perennial ryegrass was positively correlated with mean pH and also significantly ($P < 0.001$) correlated with herbage Mn

Table 5.8 Experiment 1: Regression analysis of herbage Co (y) versus herbage Mn (x) concentrations

Species	First cut		Second cut	
	Regression equation	r	Regression equation	r
Mixture	y = 0.22 + 0.001x	0.42*	y = 0.04 + 0.002x	0.71***
White clover	y = (-0.40) + 0.002x	0.76***	y = (-0.23) + 0.002x	0.54**
Italian ryegrass	y = 0.14 + 0.001x	0.38	y = (-0.28) + 0.002x	0.75***
Perennial ryegrass	y = (-0.10) + 0.003x	0.76***	y = (-0.17) + 0.003x	0.76***
Timothy	y = 0.20 + 0.002x	0.23	y = 0.13 + 0.003x	0.56*
Meadow grass	y = 0.23 + 0.003x	0.26	y = 0.42 + 0.001x	0.26
Red fescue	y = 0.07 + 0.009x	0.33	y = 0.25 + 0.005x	0.41

Level of significance of correlation coefficient (r)

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

content. These observations suggest that especially for the second cut some factor other than pH was having a greater influence on the availability of Mn and Co. This unknown factor may have been redox potential as influenced by waterlogging. Certainly the effect of impeded drainage will become more marked with time and would be expected to be seen more clearly in the second cut. In the first cut, a falling redox potential would have had less time to influence Co availability and soil acidity may have been the predominant effect at this stage. If this was the case, then this work would agree with that of West (1981) who found that drainage status has a greater influence on Co availability than pH. In addition, it would appear that some species, for example, perennial ryegrass, are more sensitive with respect to Co uptake to changes in redox potential than others (for example, white clover and timothy). However, the probability remains that these results may have been due to variation in the extent of waterlogging between treatments and may not be a true species difference.

However, it must be borne in mind that the role of waterlogging in determining the measured herbage Co contents is purely speculative. The failure to obtain the expected pH trends may be a true result, with different species reacting differently to changes in pH, e.g. perennial ryegrass may be able to extract more soil Co at high pH. However, such findings are contrary to what has been previously found. In all interpretations of the data, it must be remembered that meadow grass and red fescue never became as well established as the other species and this must have some bearing on the results.

(d) Conclusions

One major conclusion from this work is that pot trials used to study Co uptake must be set up and maintained with care. Before starting the experiment careful consideration must be given to the problem of contamination to ensure that the soil is the only source of Co. While the plants are growing, great care must be taken to ensure waterlogging does not occur. This can be partly reduced by using unmilled soil which will retain at least some soil structure and prevent soil compaction. In the work of McLaren et al (1987) watering was carried out by weight. Initially all the pots were brought to a constant water potential, known to contain enough water for the plants to grow without saturating the soil. This was maintained by weighing each soil periodically and adding sufficient water to restore the original weight. However, McLaren does not mention how he compensated for plant growth. The careful control of soil conditions in pot trials has also been discussed by Fergus and Stirk (1961) who suggest aeration must be given a large consideration to make interpretation of the data meaningful. This is even more important in studies involving Co availability where drainage status plays such an influential role.

In the absence of reliable results it is difficult to suggest a good grass mixture in relation to Co content for use on hill land or predict a pH value to which it would be safe to lime without having a detrimental effect on Co uptake.

5.4.2 Experiment 2 - Grass species field trial

The Co content of the different plant species grown at Sclenteuch (soil 3) followed the expected trends, while some grasses (i.e. Perennial ryegrass, Cocksfoot, Crested Dog's Tail, Timothy, Yorkshire Fog (1st cut only)) at Balig (soil 2) produced anomalously high Co concentrations. In the past, many workers have found that clovers contain higher Co concentrations (Mitchell et al, 1957; Voss and MacPherson, 1977; Klessa et al, 1988) than grasses and Klessa et al (1988) found herbage Co contents decreased in the order white clover > perennial ryegrass > timothy. Such trends were found at Sclenteuch but not at Balig where first cut perennial ryegrass and timothy gave Co concentrations almost double that of clover. Although this effect was reduced by the second cut, perennial ryegrass maintained a higher Co concentration than clover. In addition, other workers have failed to find that Co contents are higher in clover compared to grass (Mitchell, 1960; Andrews, 1966; McLaren et al, 1987) but this has only arisen in situations where soil Co status was regarded as being low. Comparing the Sclenteuch and Balig sites the soil having the lower Co status produced the highest Co contents in clover, while the soil regarded as being of higher Co status failed to enhance the Co concentration of clover.

The following suggestions are made to explain the high Co concentrations of some of the grasses at Balig, namely:

(i) Soil contamination

The degree of soil contamination was assessed from the herbage Fe concentrations. For both cuts, all the samples were well below 200 mg Fe kg^{-1} DM with mean (n = 11):

Table 5.9 Experiment 2: Cobalt concentrations (mg Co kg^{-1} DM) of different herbage species grown at two different sites

Grass species	Balig (soil 2)		Sclenteuch (soil 3)	
	1st cut 28/5/87	2nd cut 7/9/87	1st cut 11/6/87	2nd cut 16/9/87
Clover	0.07	0.07	0.13	0.07
Italian ryegrass	0.05	0.07	0.02	0.03
Perennial ryegrass	0.15	0.11	0.04	0.05
Cocksfoot	0.12	0.32	0.02	0.03
Crested dog's tail	0.14	0.08	0.06	0.03
Timothy	0.15	0.06	0.02	0.02
Yorkshire fog	0.10	0.04	0.02	0.04
Meadow foxtail	0.04	<0.02	-	-
Meadow grass	0.09	-	0.02	0.04
Sweet vernal	0.07	-	-	-
Poa compressor	-	-	0.03	0.04
Bent	-	0.04	0.02	0.04
Red fescue	0.07	0.05	0.02	0.03
Vetch	-	<0.02	-	-
Mean ± S.E.	0.10 ± 0.012	0.06* ± 0.009	0.04 ± 0.010	0.04 ± 0.004

*excludes cocksfoot result

First cut 92 mg Fe kg^{-1} DM ± 5.1 (±SE)

Second cut 81 mg Fe kg^{-1} DM ± 4.9

(ii) Soil variability

The experiment was set up on a "look and see" basis, so plot size was small, with no replication. The high Co herbages were all restricted to one area. For various reasons, this part of the site may have corresponded to an area where soil Co availability was different. If replicate plots had been used covering the whole site any variation in herbage Co within treatments due to variable soil Co availability would have been detected.

(iii) Maturation differences

It has been well established that herbage Co contents decline with maturity (Fleming and Murphy, 1968; Murphy, 1970). At Balig, the grass was cut down and removed in the autumn of 1986 with regrowth the following season. The first cut taken in late May corresponded to a different growth stage for each species and, hence, could have influenced Co content.

(iv) Soil fertility

The clover samples were taken from discard plots which received no fertiliser apart from drift contamination. In contrast, the grasses received an annual maintenance fertiliser treatment at the start of the growing season, causing more vigorous growth which may have increased Co uptake and indeed COSAC/SARI (1982) suggest fertiliser-N use will enhance Co availability in soils of moderate to high Co status. Hence, since the clover was maintained at a

different degree of fertility than the grasses it is difficult to make direct comparisons. At Sclenteuch, however, the clover samples were taken from grass/clover plots which were maintained at the same fertility level as the grasses.

Hence, although it is difficult to establish the main reason for unexpectedly high herbage Co concentrations of some species at Balig, it may have been due mainly to differences in maturation rates between the various grasses. Additionally, at Balig, cocksfoot taken at the second cut contained very high Co concentrations. Initially, this was thought to be due to analytical problems but when the sample was re-analysed an identical result was obtained. Soil contamination was also ruled out as the herbage Fe concentration was only 72 mg Fe kg^{-1} DM. Similar results were obtained by Mitchell et al (1957) but at only one site out of four examined. He suggested the reason may lie in the distribution of Co within the plant, with the particular part sampled for cocksfoot containing high Co contents.

The results obtained highlight some of the problems in assessing Co status from both soil and herbage analysis. For example, using the MISR/SAC (1985) method of classification, the Balig soil had a moderate Co status compared with a low soil status at Sclenteuch. Despite this, both sites produced herbage Co contents below the recommended requirement of 0.10 mg Co kg^{-1} DM for grazing ruminants and puts into question the usefulness of soil analysis. In addition, the interpretation of herbage data is not clear cut. Variations in maturation rate, soil fertility, grass species and part of plant sampled makes interpretation difficult.

For example, at Sclenteuch, the clover samples indicated an adequate Co status while all the grass samples gave Co concentrations well below the guidelines.

Although the data is difficult to interpret, a sward composed predominantly of perennial ryegrass, crested dog's tail and clover appears to be the most suitable hill land grass mixture with regard to Co content.

CHAPTER 6 - THE INFLUENCE OF N-FERTILISER ON HERBAGE COBALT CONCENTRATION

6.1 INTRODUCTION

The effect of fertiliser-N on Co uptake is unclear, with the published work giving contradictory findings. While some workers found fertiliser-N application had no influence on herbage Co content (Stewart and Holmes, 1953; Wright and Lawton, 1954; Modd, 1970) others have observed a decline in herbage Co concentrations after fertiliser-N use (Percival et al, 1955; Reith et al, 1964, 1983). In contrast, Voss and MacPherson (1977) and Klessa et al (1988) found increased sward Co concentrations when fertiliser-N was applied to a mineral soil and Reith et al (1983) found a similar result with a peat.

Little information is available to explain these conflicting results. Decreased herbage Co contents have been thought to result from clover die-back (Reith et al, 1964) or a dilution effect in which enhanced growth rate after fertiliser-N treatment exceeds Co uptake (West, 1981). COSAC/SARI (1982) attempted to explain the variable response of Co uptake to fertiliser-N application in terms of soil Co status. They suggest the application of fertiliser-N reduces herbage Co on Co-deficient or borderline soils and on naturally freely-drained soils irrespective of Co status. However, on poorly drained soils with adequate Co or on deficient soils which have been treated with Co the application of fertiliser-N may or may not lead to a change in herbage Co concentrations.

The aim of this work was to determine the reasons behind the variable response in Co uptake to fertiliser-N application and provide recommendations as to how fertiliser-N will influence herbage Co in

a particular situation. In order to carry this out, a field trial was set up in an area which had a past history of Co deficiency. Both the effect of cobalt sulphate and fertiliser-N application on herbage Co concentrations were investigated using a series of field plots. It has been suggested (Klessa et al, 1988) that the form of nitrogen used may be one of the reasons behind the contradictory findings obtained in the past. Hence, three fertiliser-N forms were used, namely ammonium nitrate, nitrochalk and urea, all known to produce a different effect on soil pH and, therefore, possibly on Co availability and uptake. For each form, different rates of application were used. In order to test the hypothesis that the availability of soil Co governs the influence of fertiliser-N on herbage Co, all the treatments were repeated on plots which had received a dressing of cobalt sulphate. Over two summers, grass samples were taken periodically and herbage Co content measured.

6.2 MATERIALS AND METHODS

6.2.1 Site

The trial site was situated at Upper Auchinlay Farm, near Dunblane, Stirlingshire (Grid Ref. No. NN775032) and on the same field as the main animal trial as detailed in Chapter 9. Details of the soil are given in Table 9.1 A 70 m x 30 m area (Fig. 9.1) was fenced off at the bottom corner of this field before it was treated with cobalt sulphate.

6.2.2 Treatments

(a) Fertiliser-N forms

Three different fertilisers were used:

(i) Ammonium nitrate (NH_4NO_3) containing 34.5% N.

(ii) Urea ($CO(NH_2)_2$) containing 46% N.

(iii) Nitrochalk (74% NH_4NO_3 + 26% $CaCO_3$) containing 26% N.

(b) Fertiliser-N rate

(i) *First year (1986)*

In the first year four rates were examined as detailed in Table 6.1. These were chosen to correspond to rates which might be used in a practical situation. At Upper Auchinlay Farm, the farmer used a fairly high rate of 87 kg N ha^{-1} applied once per year in April.

Fertiliser-N treatment rates consisted of 0, 43.5 and 87 kg N ha^{-1} applied as a single dressing before the first cut. After the first cut, these rates were repeated. In addition, to half of the plots receiving 87 kg N ha^{-1} before the first cut, no further fertiliser-N was applied (Table 6.1). There were eighteen no N control plots (of which nine had received Co) and three replicates of the remaining fertiliser-N x $\pm$ cobalt treatments.

(ii) *Second year (1987)*

In the second year a total of five rates were examined as shown in Table 6.2. From the 1986 results it was decided to examine a higher application rate than had been used before. Twelve plots (i.e. previously control no N plots in 1986, of which six had been treated with Co) received an application rate of 174 kg N ha^{-1} before each cut as either urea or ammonium nitrate. There were six no N control plots. All the other application rates remained as in 1986, but since three cuts were taken in 1987 as opposed to two in 1986, the total N applied was greater in 1987.

Table 6.1 Fertilizer N treatments at Upper Auchinlay farm in 1986

N-fertiliser form	Application (kg N ha^{-1})		Total N applied (kg N ha^{-1})
	Before the 1st cut 11/4/86	Before the 2nd cut 4/7/86	
Nitrochalk	None	None	None
	43.5	43.5	87
	87	87	174
	87	None	87
Ammonium nitrate	None	None	None
	43.5	43.5	87
	87	87	174
	87	None	87
Urea	None	None	None
	43.5	43.5	87
	87	87	174
	87	None	87

Table 6.2 Fertilizer N treatments at Upper Auchinlay farm in 1987

N-fertilizer form	Application (kg N ha^{-1})			Total N applied (kg N ha^{-1})
	Before the 1st cut 15/4/87	Before the 2nd cut 3/6/87	Before the 3rd cut 23/7/87	
Nitrochalk	None	None	None	None
	43.5	43.5	43.5	130.5
	87	87	87	261
	87	None	None	87
Ammonium nitrate	43.5	43.5	43.5	130.5
	87	87	87	261
	174	174	174	522
	87	None	None	87
Urea	43.5	43.5	43.5	130.5
	87	87	87	261
	174	174	174	522
	87	None	None	87

(c) Co treatment

To half of the 72 plots, hydrated cobalt sulphate ($CoSO_4.7H_2O$) was applied at a rate of 0.6 kg Co ha^{-1}. The elemental analysis of the cobalt sulphate used is given in Table 6.3. This was applied once as a spray on 22/4/86 following the first dressing of the plots with fertiliser-N. As the cobalt sulphate contained insoluble impurities which might have blocked the sprayer the solution was first filtered through Whatman No. 1 filter paper.

6.2.3 Experimental plan

Twenty-four treatments detailed in Table 6.1 and 6.2 were replicated three times to give 72 plots. The experiment was arranged as a randomised block design of three blocks each containing 24 treatments in a 6 x 4 arrangement. Each plot was 3 m x 2 m with a 1 m discard between plots and a 2 m discard between blocks. A 6 m discard was left between the blocks and the field fence (Fig. 6.1).

6.2.4 Sampling and analysis

(a) Soil

Soil samples were taken at the end of each year (20/8/86 and 31/8/87) by the methods detailed in Chapter 3. Soil pH was determined on air-dried soil (see Chapter 3).

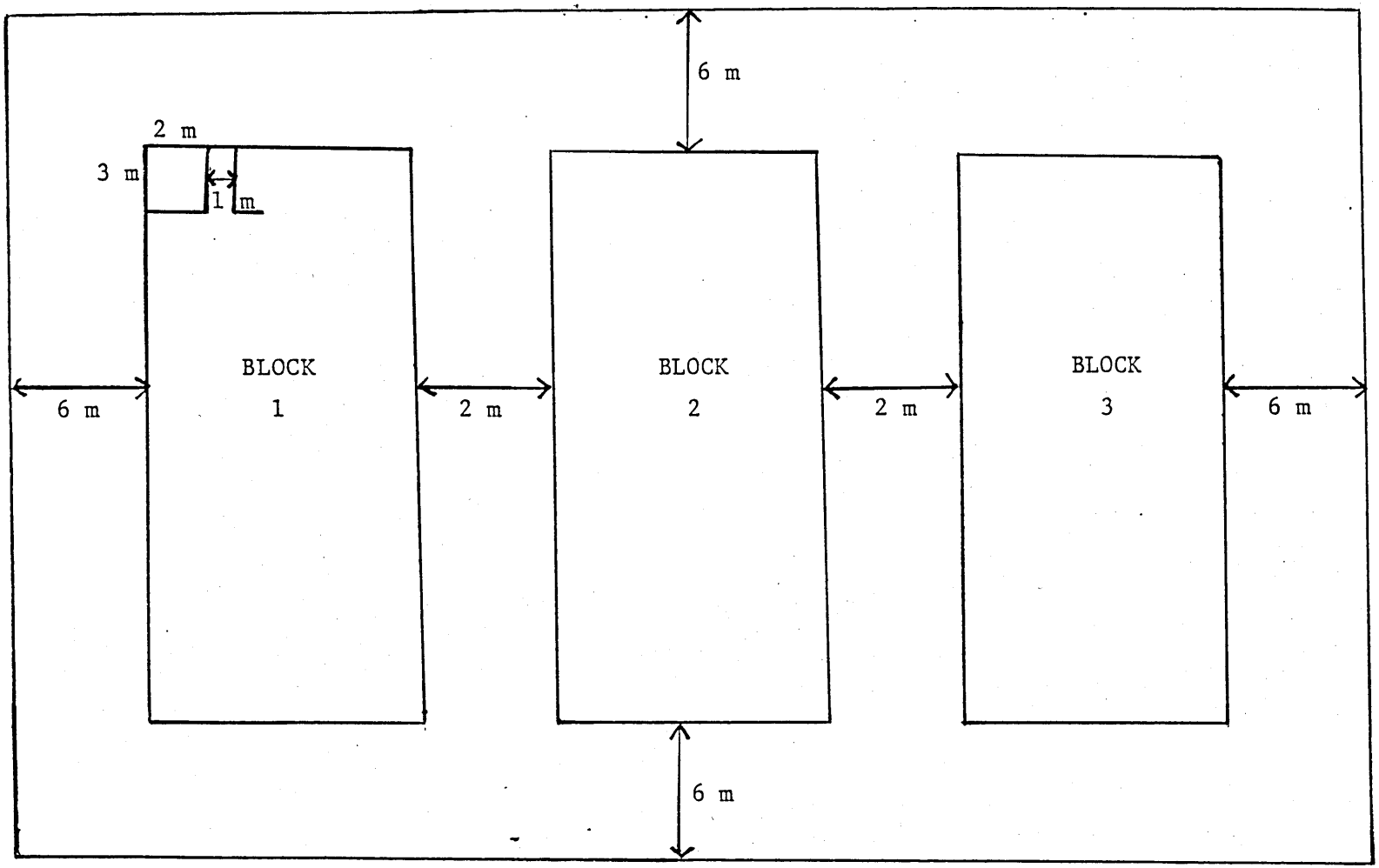
(b) Herbage

Representative herbage samples were taken each year by the methods described in Chapter 3. After the first cut in 1986, and the first and second cut in 1987 all the remaining grass was cut using an "Agria" mower and discarded. At the end of the first year, the fence was lowered allowing sheep access

Table 6.3 Elemental composition of cobalt sulphate applied at Upper Auchinlay farm

Element	Composition (mg kg^{-1})
Co	210,000
Ni	400
Fe	100
Cu	60
Zn	20
Na	200
Mg	50

Figure 6.1 Experimental Layout of Field Plot Experiment to examine the effect of Fertiliser N on Herbage Co contents



to graze down the area during the winter months and, therefore, provide a short cropped pasture for the start of the trial the following year.

In 1986, two cuts were taken (30/6/88 and 18/8/86), while in 1987, three cuts were taken (1/6/87, 20/7/87 and 31/8/87) reflecting the better growing season. All herbage samples were analysed for Co, Fe, Mn, Mo, Cu and Zn as described in Chapter 3.

In addition, in 1987 the % clover present in the sward was estimated. This involved the use of a 30 cm x 30 cm template placed at random within each plot and % clover was then estimated.

No measure of production response was taken.

6.3 RESULTS

For all cuts, soil contamination was negligible with herbage Fe concentrations well below 200 mg Fe kg^{-1} DM (Table 6.4).

Herbage Co concentrations in 1987 were very low ranging between 0.02-0.04 mg Co kg^{-1} DM (Table 6.5 (a) and (b)). Such concentrations are very close to the detection limit of the analytical method used for Co determination.

Throughout the two year period cobalt sulphate treatment of pasture maintained a significantly ($P < 0.001$) greater herbage Co concentration than untreated pasture (Table 6.5). In 1986, Co treatment produced herbage with two to twelve times more Co than untreated pasture and maintained herbage Co concentrations above the recommended lower limit for grazing ruminants (i.e. 0.10 mg Co kg^{-1} DM). The only exception to this was found on the Co-treated plots

Table 6.4 Mean herbage Fe concentrations (mg Fe kg^{-1} DM) of each cut (± S.E.) (n = 72)

Year	Cut	Herbage Fe (mg Fe kg^{-1} DM)
1986	First	79 ± 1.4
	Second	93 ± 2.0
1987	First	66 ± 1.6
	Second	82 ± 1.4
	Third	89 ± 1.5

Table 6.5 (a) Influence of fertilizer N and Co application on the mean Co content of herbage (mg Co kg^{-1} DM) (n = 3)

(a) 1986

N-fertilizer form	Total N-applied (kg N ha^{-1})	1st cut		2nd cut	
		Co treatment		Co treatment	
		None	0.6 kg Co ha^{-1}	None	0.6 kg Co ha^{-1}
Nitrochalk	0	0.033	0.457	0.047	0.193
	87	0.023	0.323	0.030	0.113
	174	0.030	0.273	0.030	0.133
	87**	0.030	0.273	0.047	0.140
Urea	0	0.040	0.280	0.047	0.153
	87	0.040	0.217	0.037	0.123
	174	0.035	0.238	0.030	0.067
	87**	0.035	0.238	0.050	0.120
Ammonium nitrate	0	0.043	0.480	0.047	0.177
	87	0.033	0.343	0.043	0.220
	174	0.037	0.330	0.040	0.133
	87**	0.037	0.330	0.040	0.200
S.E.		0.0499		0.0271	

**applied at start of grazing season only

Table 6.5 (b) Influence of fertilizer N and Co application on mean Co content of herbage (mg Co kg^{-1} DM) (n = 3)

(b) 1987

N-fertilizer form	Total N-applied (kg N ha^{-1})	1st cut		2nd cut		3rd cut	
		Co treatment		Co treatment		Co treatment	
		None	0.6 kg Co ha^{-1}	None	0.6 kg Co ha^{-1}	None	0.6 kg Co ha^{-1}
Nitrochalk	0	0.020	0.040	0.027	0.037	0.027	0.033
	130.5	0.013	0.027	0.013	0.027	0.013	0.037
	261	0.017	0.025	0.020	0.027	0.013	0.033
	87**	0.017	0.025	0.027	0.043	0.027	0.034
Urea	130.5	0.023	0.037	0.023	0.043	0.020	0.037
	261	0.020	0.037	0.027	0.027	0.020	0.037
	522	0.023	0.040	0.027	0.033	0.023	0.027
	87**	0.023	0.040	0.020	0.030	0.027	0.026
Ammonium nitrate	130.5	0.017	0.017	0.017	0.037	0.021	0.040
	261	0.025	0.027	0.027	0.027	0.023	0.037
	522	0.033	0.048	0.027	0.053	0.023	0.060
	87**	0.033	0.048	0.013	0.020	0.020	0.033
S.E.		0.0062		0.0055		0.0066	

**applied at start of grazing season only

receiving 174 kg N ha^{-1} as urea, where herbage Co content fell to 0.07 mg Co kg^{-1} DM at the second cut. Only one year after application the herbage Co content had fallen dramatically to around 25-60% of that recorded at the second cut in 1986 but in general remained significantly ($P < 0.001$) higher than that obtained for untreated pasture (Table 6.5 (b)). Nevertheless, the actual level was below that recommended for grazing ruminants.

Although fertiliser-N form, in general, had no significant influence on herbage Co contents, in both years the application of ammonium nitrate and to some extent urea produced herbage of higher Co concentrations than did identical amounts of N as nitrochalk.

On average in 1986, plots receiving fertiliser-N produced herbage with a lower Co content than the no N controls. However, there did not appear to be a relationship between the decline in herbage Co concentrations and fertiliser-N application rate. In some instances, the lower application rate of 43.5 kg N ha^{-1} before each cut, had a greater effect in reducing herbage Co contents than a similar treatment with 87 kg N ha^{-1}, while in others the reverse was seen, but in all cases the differences were non-significant. In 1987, fertiliser-N application produced a more variable response in Co uptake. However, the yearly application of 522 kg N ha^{-1} as ammonium nitrate in 1987 to the Co treated plots produced herbage having significantly ($P < 0.05$) higher Co concentrations than the no N controls. However, when similar amounts of N were applied as urea no such effect was seen.

In both years, neither fertiliser-N form nor application rate had any significant effect on soil pH compared with the no N controls (Table 6.6 (a) and (b)) with one exception. A yearly application

Table 6.6 (a) Influence of fertilizer N on mean soil pH measured on air-dried soil sampled on 20/8/86 (n = 3)

N-fertilizer form	Total N-applied kg N ha^{-1}	pH
Nitrochalk	0	6.1
	87	6.2
	174	5.9
	87**	6.0
Urea	0	6.0
	87	6.0
	174	6.2
	87**	6.0
Ammonium nitrate	0	6.1
	87	5.9
	174	6.0
	87**	5.9
S.E.		0.09

**applied at start of grazing season only

Table 6.6 (b) Influence of fertilizer N on mean soil pH measured on air-dried soil sampled on 31/8/87 (n = 3)

N-fertilizer form	Total N-applied kg N ha^{-1}	pH
Nitrochalk	0	5.9
	130.5	5.9
	261	5.6
	87**	5.7
Urea	130.5	5.8
	261	5.8
	522	5.2
	87**	5.6
Ammonium nitrate	130.5	5.8
	261	5.7
	522	5.2
	87**	5.8
S.E.		0.09

**applied at start of grazing season only

of 522 kg N ha^{-1} as either urea or ammonium nitrate resulted in a significant ($P < 0.001$) reduction in soil pH compared with the no N controls. In general, all the pH values measured in 1987 were on average 0.3 pH unit below those seen in 1986, irrespective of the form of N used.

The application of N, irrespective of form, significantly reduced ($P < 0.001$) the clover content of the sward (Table 6.7) and for some treatments this resulted in an almost total elimination of clover.

6.4 DISCUSSION

6.4.1 Cobalt sulphate treatment

Cobalt sulphate application resulted in significantly ($P < 0.001$) greater herbage Co concentrations in both years, but produced herbage containing >0.10 mg kg Co DM^{-1} only in the first year. The reasons for and the consequences of this poor residual value are discussed in Chapter 9.

In 1986, the first cut samples from all the Co-treated plots contained very high Co concentrations (>0.2 mg Co kg DM^{-1}) (Table 6.5), as a result of foliar contamination. Similar results were obtained for the first cut following Co application by Klessa et al (1988) who also found that herbage Co concentrations fell in the later cuts as the contaminated herbage was removed or Co adhering to the leaves was washed into the soil.

6.4.2 Fertiliser nitrogen form

In both years, fertiliser-N form had no significant influence on herbage Co concentrations, but in general the application of ammonium nitrate and to a lesser extent urea produced

Table 6.7 Influence of fertilizer N and Co application on mean % clover in the sward in 1987 (n = 3)

N-fertilizer form	Total N-applied (kg N ha^{-1})	1st cut		2nd cut		3rd cut	
		Co treatment		Co treatment		Co treatment	
		None	0.6 kg Co ha^{-1}	None	0.6 kg Co ha^{-1}	None	0.6 kg Co ha^{-1}
Nitrochalk	0	63.3	48.3	50.0	50.0	35.0	43.3
	130.5	5.2	4.0	1.0	2.3	0.3	0.7
	261	7.0	4.4	0.3	0.3	0	0
	87**	7.0	4.4	20.7	8.3	15.0	3.3
Urea	130.5	5.2	6.7	2.3	3.7	3.7	4.0
	261	17.2	15.7	1.0	0.7	0.3	1.7
	522	21.7	6.0	0.7	0	0.3	0.2
	87**	21.7	6.0	36.7	8.3	8.3	7.7
Ammonium nitrate	130.5	5.3	3.5	1.0	7.0	0.7	1.8
	261	7.0	6.0	0	0	1.7	0.7
	522	8.3	5.3	0.3	1.0	0.3	2.3
	87**	8.3	5.3	6.7	17.3	3.7	5.0
S.E.		7.041		5.331		2.007	

**applied at start of grazing season only

herbage containing more Co than did those plots treated with nitrochalk. The majority of workers have not examined the effect of different fertiliser-N forms on Co uptake but concentrated instead on how one type influences herbage Co concentrations. However, Klessa et al (1988) found results similar to those reported here in that higher herbage Co contents were recorded when ammonium nitrate as opposed to nitrochalk fertilisers were used. Further, Klessa et al (1988) suggested this may be due to soil acidification by ammonium containing fertilisers enhancing Co availability, while the presence of lime in nitrochalk compensates for this effect. In contrast, urea, although causing an initial alkalising effect from the production of ammonia, is an acidifying fertiliser. Hence, soil acidification resulting from both ammonium nitrate or urea use may account for the slightly higher herbage Co concentrations obtained with these fertilisers than those treated similarly with nitrochalk. However, soil pH measured after the last cut in 1986 and 1987 showed no significant differences between the three fertiliser-N forms, with the drop in pH between 1986 and 1987 approximately 0.3 pH unit irrespective of the fertiliser-N form used. Klessa et al (1988) also found no significant differences in soil pH following fertiliser-N application as either nitrochalk or ammonium nitrate until after three growing seasons, when plots treated with ammonium nitrate showed a fall of 0.3 pH unit compared with the nitrochalk treated plots. This was more marked for the higher fertiliser-N application rates and the difference widened in subsequent years.

In addition, application of fertiliser-N as ammonium nitrate, urea or nitrochalk produced an almost identical reduction in the clover content of the sward, and hence differences in % clover content between the different forms cannot explain the observed trends.

6.4.3 Rate of fertiliser-N application

In general, the application of fertiliser-N in 1986, irrespective of whether the pasture had been treated with Co, produced a reduction in herbage Co content while in 1987 a more variable response was observed. However, in 1987 herbage Co concentrations were generally close to the detection limit of the analytical method used in this study. Hence, differences between treatments could have arisen from analytical variations rather than actual treatment effects. Other workers (Percival et al, 1955; Reith et al, 1964, 1983) also obtained a decline in Co concentrations following fertiliser-N application. In addition, Reith et al (1964) suggested that clover die-back may be the principal reason behind the lowering of sward Co contents after fertiliser-N treatment. This would also appear to be the case at Upper Auchinlay Farm, where the application of nitrogen virtually eliminated clover from a mixed sward. If clover contains a higher concentration of Co than grasses (Voss and MacPherson, 1977) such a dramatic lowering of clover content will decrease the overall Co content of a sward.

In contrast to the results obtained here, Voss and MacPherson (1977) and Klessa et al (1988) found that fertiliser-N application increased herbage Co concentrations by as much as 100% when 80 kg N ha^{-1} as opposed to 17.5 kg N ha^{-1} per cut was applied.

However, in their work, although herbage Co concentrations were classed as deficient, they were still greater than those of untreated pasture at Upper Auchinlay Farm. Therefore, as suggested by COSAC/SARI (1982) the influence of fertiliser-N may vary depending on soil Co status. However, this appears to be in conflict with results found at Upper Auchinlay Farm, where fertiliser-N application to Co-treated pasture in 1986 still resulted in a lowering of the Co content of the sward compared with the no N controls. This is in contrast to the findings of Reith et al (1983), who found fertiliser-N application to Co-treated plots resulted in higher herbage Co concentrations than in no N controls, despite similar N treatments to plots receiving no Co showing a decline in herbage Co contents. In light of the information available no reasons can be given to explain these findings.

In both years, applying a single dressing of 87 kg N ha^{-1} as nitrochalk at the start of the growing season as opposed to a split 43.5 + 43.5 kg N ha^{-1} (applied before each cut) produced herbage with a higher Co concentration. However, this was not always the case for similar fertiliser application rates using urea or ammonium nitrate. These findings could have practical implications. For example, a farmer using nitrochalk as his nitrogen source will produce a smaller effect on herbage Co contents in mid to late summer, if he uses a single dressing at the start of growth in early spring. At this time with a low plant density and height, Co intake by the grazing ruminant is enhanced by soil ingestion (Thornton, 1973) and this will compensate for the reduction in Co content of the sward following fertiliser-N application. However, it must be bourne in mind that a single spring dressing will enhance growth in early summer,

while a split dressing will also give a flush of growth in late summer/early autumn. (Cooke, 1983). On some farms a late boost of grass growth may be advantageous (for example, when weaned spring lambs are being finished for sale) and a split dressing may be the most cost-effective method of applying N. In such situations, where fertiliser-N application reduces herbage Co uptake, some form of animal Co supplementation may be required to ensure maximum production.

Since the differences in herbage Co contents between an application of 43.5 or 87 kg N ha^{-1} before each cut were non-significant, no conclusions can be made as to how the rate of fertiliser-N application influences herbage Co concentrations. Previous workers (Reith et al, 1983, and Klessa et al, 1988) found a linear relationship between the content of Co in herbage and fertiliser-N application rate, when they used a larger number of fertiliser rates covering a wider range.

Whereas the results reported here have tended to suggest that herbage Co concentrations decline following fertiliser-N application, all the Co-treated plots which received 174 kg N ha^{-1} as ammonium nitrate before each cut in 1987 produced higher herbage Co concentrations than the no N controls. Since these increases were as high as 80% at the third cut, their consistency cannot be ascribed to analytical errors. However, identical dressings with urea failed to increase herbage Co contents above the no N controls. While the application rates of fertiliser-N used in 1986 resulted in no significant effect on soil pH compared with the no N controls, in 1987 a dressing of 174 kg N ha^{-1} after each cut as urea or ammonium nitrate significantly ($P < 0.001$) lowered the

soil pH below the no N controls. This corresponded to a drop of approximately 0.8 pH unit from the values measured on these plots in 1986 compared with the decline of around 0.3 pH unit recorded for all the other treatments. Such a reduction in soil pH would enhance Co availability and hence plant uptake, but if acidity was the sole explanation then similarly increased herbage Co concentrations would have been expected for both the ammonium nitrate and urea treated plots. One possible explanation may lie in the form of nitrogen taken up by plants. It has been established that the presence of NH_4-N reduces the uptake of other cations such as Ca^{2+} and Mg^{2+} (Cooke, 1983) but no work appears to have been conducted into this phenomenon in terms of trace element uptake. In the absence of nitrification, ammonium nitrate containing fertilisers will supply both NH_4-N and NO_3-N, while urea will produce only NH_4-N. Under such circumstances the possible antagonism between Co^{2+} and NH_4^+ could result in a greater uptake of Co in the presence of ammonium nitrate, where a smaller proportion of the applied nitrogen is present as NH_4-N. This may also be the case, even in soils which do nitrify immediately after fertiliser-N application when nitrification has not had time to occur. However, the highest N application rate used in 1987, i.e. 522 kg N ha^{-1} whether as urea or ammonium nitrate produced little effect on herbage Co concentrations when applied to soil which had received no Co. Following Co application to soil, Co will be retained on the exchange sites. Thus for a given fall in soil pH, more Co will be released from the soil which has received the Co dressing than from the one not treated and, hence, bring about a greater effect on Co uptake.

Hence, fertiliser-N application has a variable influence on herbage Co concentrations, being dependent on the rate of application, the type of fertiliser used and the Co status of the soil. Thus, it appears that general recommendations cannot be made as to how fertiliser-N will affect herbage Co contents, with each situation showing a different response. However, in general, it can be stated that ammonium nitrate will produce herbage of higher Co concentration than nitrochalk.

6.4.4 Other elements

Since Co was the main element of interest only a brief account will be given of the influence of fertiliser-N on the herbage concentrations of Fe, Mn, Cu, Zn and Mo. In both years, herbage Zn and Cu contents increased with increased fertiliser-N application rates, similar to the findings of Mudd (1970). However, fertiliser-N application had a variable effect on Fe, Mn and Mo concentrations in herbage and no consistent trends were seen.

CHAPTER 7 - THE RELATIONSHIP BETWEEN COBALT STATUS AND IMMUNOCOMPETENCE AND AN ASSESSMENT OF THE VARIOUS BLOOD CRITERIA USED TO DIAGNOSE COBALT DEFICIENCY IN CATTLE

7.1 INTRODUCTION

Previous workers (MacPherson et al, 1976) have demonstrated an enhanced susceptibility to infection in Co-deficient sheep. It was thought that the higher incidences of skin bruising, enteric and respiratory problems with Co depletion was associated with an induced ascorbic acid deficiency. However, more recently, the low immunocompetence of neutrophils found in Co-deficient ruminants has been suggested for this increased vulnerability to disease (Wright et al, 1982; Fisher and MacPherson, 1986; MacPherson et al, 1987). Further, it has been proposed that such effects may have a major bearing on animal productivity resulting in a low viability of lambs born to Co-deficient ewes (Fisher and MacPherson, 1986) and a greater vulnerability to internal parasitic infection (Wright et al, 1982; MacPherson et al, 1987).

Despite the many problems involved in both the determination and interpretation of serum vitamin B_{12} values, the use of vitamin B_{12} remains the most commonly used diagnostic parameter for Co deficiency. Factors such as prolonged yarding, diseases resulting in liver damage (Clark and Millar, 1983) and starvation (Miller et al, 1984) have been identified as causing 'falsely' elevated serum vitamin B_{12} values. In addition, large animal variations within a flock make diagnosis difficult when only a limited number of samples are available (Somers and Gawthorne, 1969). The problem of measuring vitamin B_{12} is thwarted by the lack of standard methods (Mills,

1981) and the poor understanding of how much of the vitamin B_{12} measured is composed of inactive analogues (Millar and Penrose, 1980). The latter is a particular problem with cattle where up to fifty percent of the total vitamin B_{12} content of serum may be made up of inactive analogues (Halpin et al, 1984). Various other diagnostic methods have been investigated, such as urinary formiminoglutamic acid (Stebbings and Lewis, 1986) and urinary methyl malonic acid (Millar and Lorentz, 1979), but have been dismissed due to either analytical or interpretative problems. However, more recently, a sensitive method of determining MMA in serum or plasma has been developed (McMurray et al, 1986). This has shown some promise as a diagnostic tool in sheep (Fisher and MacPherson, 1986; Rice et al, 1987) but has received no attention in relation to cattle.

Two housed trials using calves were established to investigate both the influence of Co status on the immune response of cattle and to assess the merits of various diagnostic criteria, including serum MMA and vitamin B_{12} (by radio-assay and microbiological techniques). In Experiment 1, six calves were kept on a Co-deficient diet for between eight to sixteen months before undergoing Co repletion. The methods used included weekly dosing with Co or the administration of a slow-release form of Co either as a commercial bolus or as the more novel Co "needle". In a similar manner, in Experiment 2, ten calves were fed a Co-deficient diet but five received regular Co supplementation as fortnightly vitamin B_{12} injections throughout the ten month experiment. In addition, Experiment 3 examined the influence of Co supplementation on the severity of Ostertagia ostertagi infection in cattle using the animals from Experiment 2.

7.2 MATERIALS AND METHODS

7.2.1 Diet and ration formulation

For all experiments, a Co-deficient ration containing $\leq$0.04 mg Co kg DM^{-1} was fed. In Experiment 1, this consisted of timothy hay (0.06 $\pm$ 0.01 mg Co kg DM^{-1}), micronised maize (0.026 $\pm$ 0.005 mg Co kg DM^{-1}) and prairie meal (0.03 $\pm$ 0.009 mg Co kg DM^{-1}). A similar diet was used for Experiment 2 during the first six months, after which the protein source was changed from prairie meal to urea. It was assumed that urea was low in Co as found by Mills (1981). This changeover was carried out since a new batch of prairie meal received at this time contained anomalously high Co concentrations. This new diet was used throughout the remainder of Experiment 2 and the whole of Experiment 3. In addition, appropriate amounts of salt and dicalciumphosphate were added to all the rations to overcome dietary deficiencies of sodium, calcium and phosphorus.

All the rations were formulated using the BEEFRAT computer programme as developed by the "Agricultural Chemistry Division", West of Scotland College, to produce a diet balanced for protein and energy. In order to maintain this balanced diet, regular samples of the various dietary constituents were submitted to the "Analytical Services Unit", West of Scotland College, for routine dietary analysis as detailed in Chapter 3. For Experiment 1, when liveweights were <350 kg, the ration aimed to produce a daily liveweight gain (DLWG) of around 0.7 kg, but at animal weights >350 kg this was increased to 1.0 kg per day. On the other hand, during Experiments 2 and 3 the ration was formulated to give a DLWG of 0.7 kg.

7.2.2 Experimental plan

(a) Experiment 1 - The effect of Co depletion and repletion on the immunocompetence and Co status of cattle

Six Friesian castrates initially 12 weeks old were maintained on a Co-deficient diet as detailed in 7.2.1 for 73 weeks. During the first 55 weeks the calves were pair penned at the "Metabolism Unit", West of Scotland College, after which they were all put into a single slatted pen at the "Brickrow Farm Unit", West of Scotland College. After 36 weeks, two of the animals (hereafter called Group 2) were repleted using an oral Co drench with occasional vitamin B_{12} injections (neo-cytamen from Glaxovet) (Table 7.1). A further two animals (hereafter called Group 1) at week 59 were given a slow-release Co form. One animal was given a "Cosecure" slow-release bolus (U.K. Wellcome Foundation Ltd.), the other one "Cobalt needle" (Beechams). A further "Cobalt needle" was given to this animal at week 64. The remaining two animals (hereafter called Group 3) were kept on the depletion diet for the complete trial. In addition, all six calves received oral doses of selenium (as sodium selenite) when the concentration of the Se containing enzyme, GSH-Px, in whole blood was indicative of Se deficiency (<15 U ml^{-1} cells at 30°C). Doses were given as 5 mg Se per head at week 22 and 10 mg Se per head at weeks 26, 38 and 60.

(b) Experiment 2 - A comparison of immunocompetence and Co status of Co supplemented and depleted cattle

Ten, 12 week old Friesian castrates were fed a Co-deficient diet for 43 weeks as detailed in 7.2.1 During the first

Table 7.1 Co repletion using Co drench and occasional vitamin B12 injections

(a) Oral Co drench

Week no.	Amount of Co administered (mg) as a Co $SO_4.7H_2O$ drench
36	10
38	260
Weekly between 39-49	125
Weekly between 51-73	10

(b) Vitamin B_{12} injections

Week no.	Vitamin B_{12} (μg) administered
55, 58	1000
36, 63, 64, 67, 68	2000
38	4000

35 weeks, the calves were penned individually at the "Metabolism Unit", West of Scotland College, before being transferred to one large slatted pen at "Brickrow Farm Unit", West of Scotland College. Throughout the trial, five of the cattle received Co supplementation initially as an oral 10 mg Co drench of $CoSO_4.7H_2O$ at week 2 and a "Cosecure" slow-release bolus (U.K. Wellcome Foundation Ltd.) at week 3, followed by regular fortnightly 2000 µg vitamin B_{12} injections (neo-cytamen, Glaxovet) from week 10 onwards. After 16 weeks one animal from the depleted group had to be destroyed due to a severe viral infection. This animal was replaced with one of similar weight and age, and introduced to the Co-deficient diet at week 20. However, all the results from this animal were excluded from any statistical analysis since low serum vitamin B_{12} values (50 ng l^{-1}) were not obtained until week 39.

(c) Experiment 3 - Effects of Co status on the degree of severity of Ostertagia ostertagi infection

This trial was carried out on the same ten animals (approximately one year old) as were used in Experiment 2, with the cattle sub-divided into four treatment groups, namely:

A CONTROL - no parasitic infection

Group 1 - 2 animals from Co supplemented group

Group 2 - 2 animals from Co depleted group

B INFECTED

Group 3 - 3 animals from Co supplemented group

Group 4 - 3 animals from Co depleted group

In order to eliminate the danger of cross-infection, the control and infected animals were housed in separate but

adjacent slatted pens at "Brickrow Farm Unit". Co supplementation was given as fortnightly 3000 μg vitamin B_{12} injections with the parasitic infection administered daily as 5000 Ostertagia ostertagi stage 3 larvae (as supplied by Glasgow University Veterinary School) per head to the appropriate animals for a total of eight weeks. On the day following the final parasitic treatment, all the infected animals were given a cobaltised anthelmintic (Endozol containing 3.75% albendazole + 2.7% cobalt sulphate, Robert Young and Co Ltd.). The dosing regime was based on liveweight and according to the manufacturer's instructions.

7.2.3 Parameters measured

Details of all the analytical procedures are given in Chapter 3.

(a) Experiments 1 and 2

For both trials, fortnightly measurements were made as below:

(i) *Co status*. Vitamin B_{12} (by microbiological assay for both experiments and also by radio-assay in Experiment 2 only) and MMA.

(ii) *Animal performance*. Liveweight (in experiment 1 weekly measurements were made from week 36 onwards).

(iii) *Immune response*. By the neutrophil function test.

In addition, total haemoglobin, whole blood GSH-Px, plasma glucose, alkaline phosphatase, aspartate transferase, Ca, Mg and Cu were monitored fortnightly during Experiment 1 and monthly in Experiment 2. Further, fortnightly measurements of serum urea concentrations were made from week 30 in Experiment 2, after the introduction of urea into the diet.

(b) Experiment 3

Weekly assessments were made of Co status and animal performance, as described for Experiment 2, with the extent of parasitic infection monitored using weekly pepsinogen and gastrin measurements and faecal worm egg counts. Additionally, serum inorganic phosphorus concentrations were determined weekly and the neutrophil function test carried out fortnightly. As in Experiment 2, monthly measurements were made of urea, GSH-Px, total haemoglobin and plasma Ca, Mg and Cu concentrations. After administration of the anthelmintic, blood samples were taken from the infected groups at 0, 1, 3, 5, 7 hours and 1, 2, 3, 5 and 7 days post treatment for serum vitamin B_{12} (microbiological assay only), MMA, pepsinogen and gastrin determination with inorganic P measured on day 7 only. Liveweight was also measured after 1, 2, 3, 5 and 7 days and faecal worm egg counts after 5 days. Further blood samples were taken 26 and 35 days after anthelmintic treatment for assessment of pepsinogen concentrations.

7.3 RESULTS

Throughout all the experiments total haemoglobin, plasma glucose, alkaline phosphatase, aspartate transferase, Ca, Mg and Cu concentrations remained within normal limits and were unaffected by treatment. In Experiment 1, whole blood GSH-Px contents gave values indicative of marginal Se deficiency on four occasions. This was remedied by oral Se treatment as detailed in 7.2.2 (a). Selenium status remained adequate during Experiments 2 and 3. Serum urea concentrations monitored during Experiment 2 and the whole of Experiment 3 indicated that the amounts of urea included in the diet did not give rise to toxicity problems.

7.3.1 Experiment 1 - The effect of Co depletion and repletion on the immunocompetence and Co status of cattle

During the first 36 weeks of the experiment a DLWG of 0.7 kg was sustained (Fig. 7.1 (a)), despite mean serum vitamin B_{12} levels falling from around 200 ng l^{-1} to 80 ng l^{-1}. Mean serum vitamin B_{12} concentrations fell to 150 ng l^{-1} after 10 weeks, with another decrease to around 100 ng l^{-1} after 14 weeks, remaining at this concentration with only minor fluctuations until week 30 when values declined further to approximately 80 ng l^{-1} (Fig. 7.2 (a)). Mean serum MMA concentration, on the other hand, after 14 weeks increased from 1.5 µmole l^{-1} to around 3 µmole l^{-1} and showed little further change during the next 22 weeks (Fig. 7.3 (a)). The immune status as measured by the neutrophil function test fell from an initial value of 35% to 15% by week 18 (Fig. 7.4 (a)), in parallel with the decline in vitamin B_{12} concentrations and in contrast to the increase in MMA values (Fig. 7.5 (a)).

Co repletion at week 36 and 59 produced no effect on liveweight performance compared with the untreated controls (Fig. 7.1 (b)), despite causing increased serum vitamin B_{12} concentrations. Regular treatment with Co oral drenching from week 36 increased serum vitamin B_{12} levels to around 200 ng l^{-1} after 4 weeks (Fig. 7.2 (b)), while serum MMA contents remained elevated at 3 µmole l^{-1} for a further 5 weeks, before falling to <1.0 µmole l^{-1} 11 weeks after repletion commenced (Fig. 7.3 (b)). However, treatment with Co in a slow-release form at week 59 resulted in a dramatic rise in serum vitamin B_{12} values within a few days of treatment to >200 ng l^{-1} (Fig. 7.2 (b)), and a drop in serum MMA concentrations to <1.0 µmole l^{-1} (Fig. 7.3 (b)). In later weeks, although serum vitamin B_{12} levels fell to around 150 ng l^{-1}, MMA

Figure 7.1 (a) Experiment 1 - Mean liveweights to week 36 (n = 6)

Average S.E. = ±6

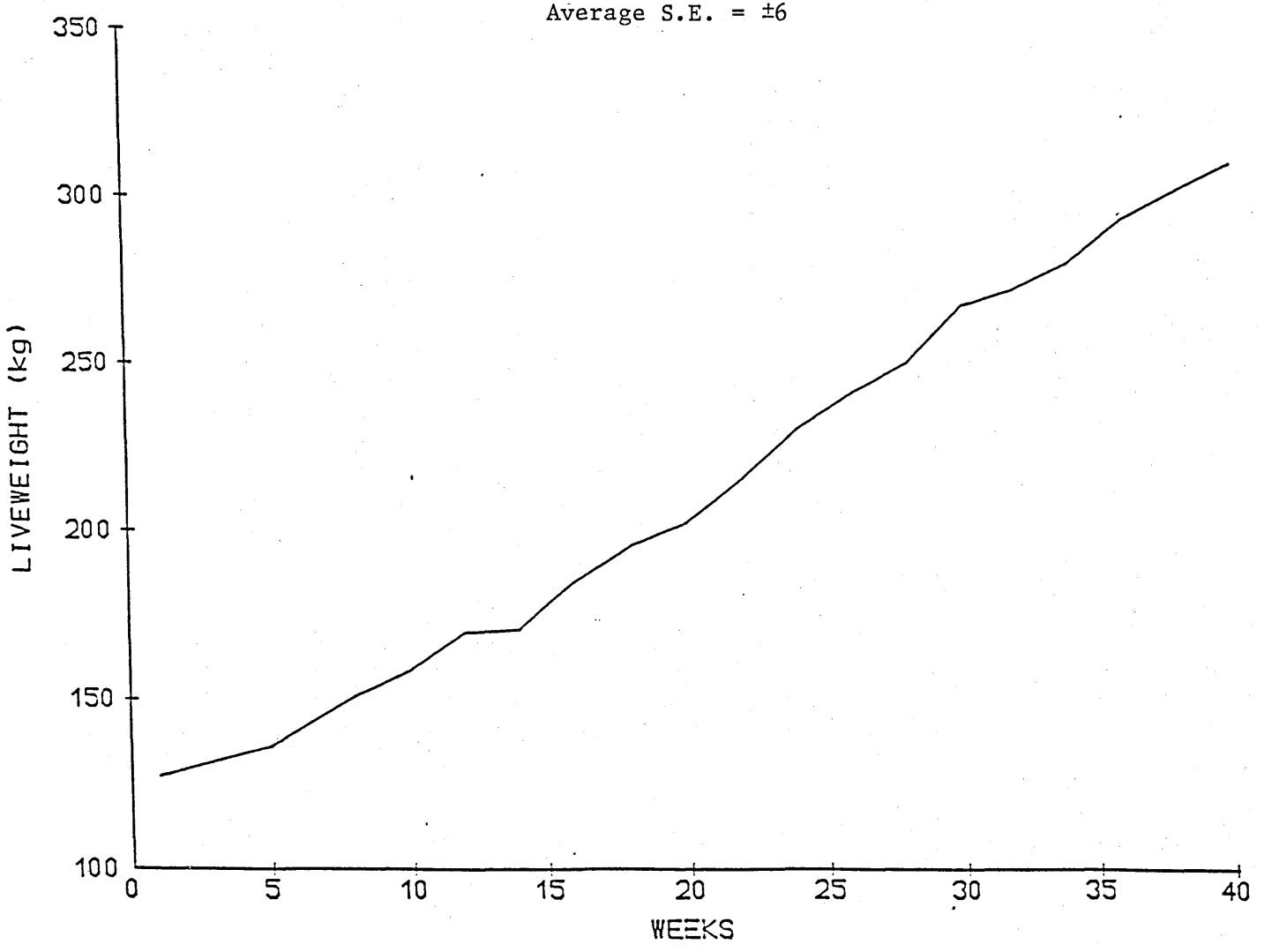


Figure 7.1 (b) Experiment 1 - Liveweights from week 36 to 73

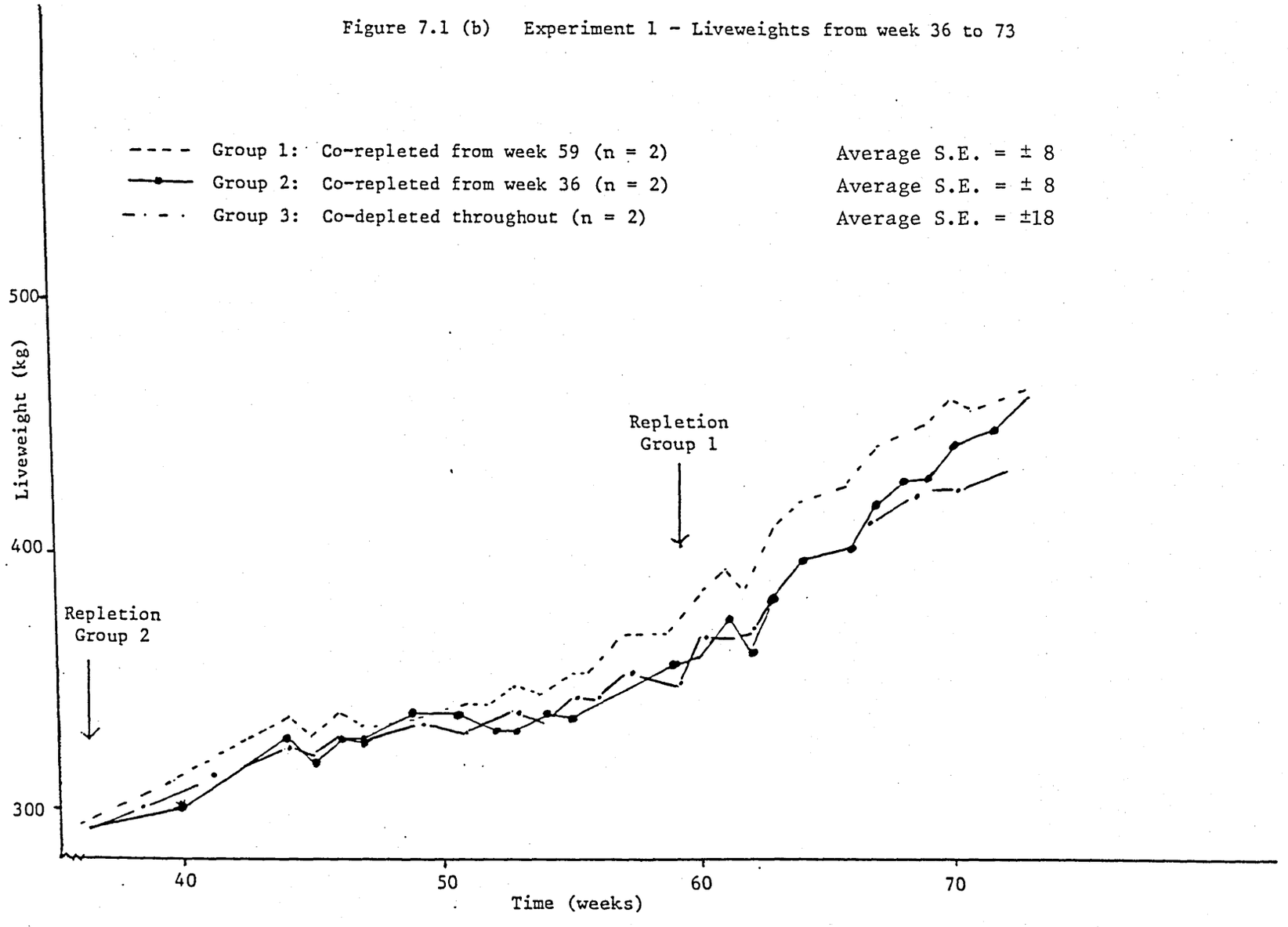


Figure 7.2 (a) Experiment 1 - Mean serum vitamin B_{12} concentrations to week 36 (n = 6)

Average S.E. = ±12

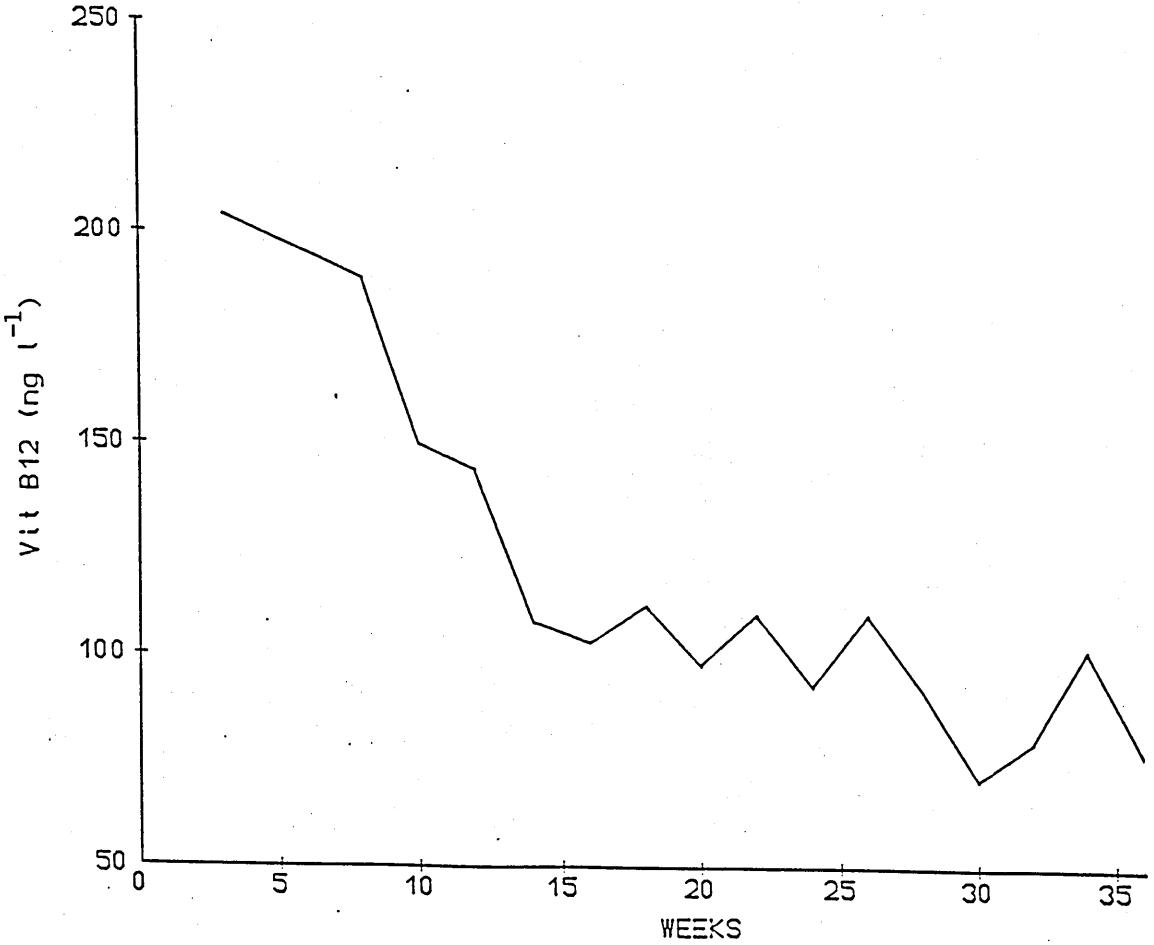


Figure 7.2 (b) Experiment 1 - Serum vitamin B_{12} concentrations from week 36 to 73

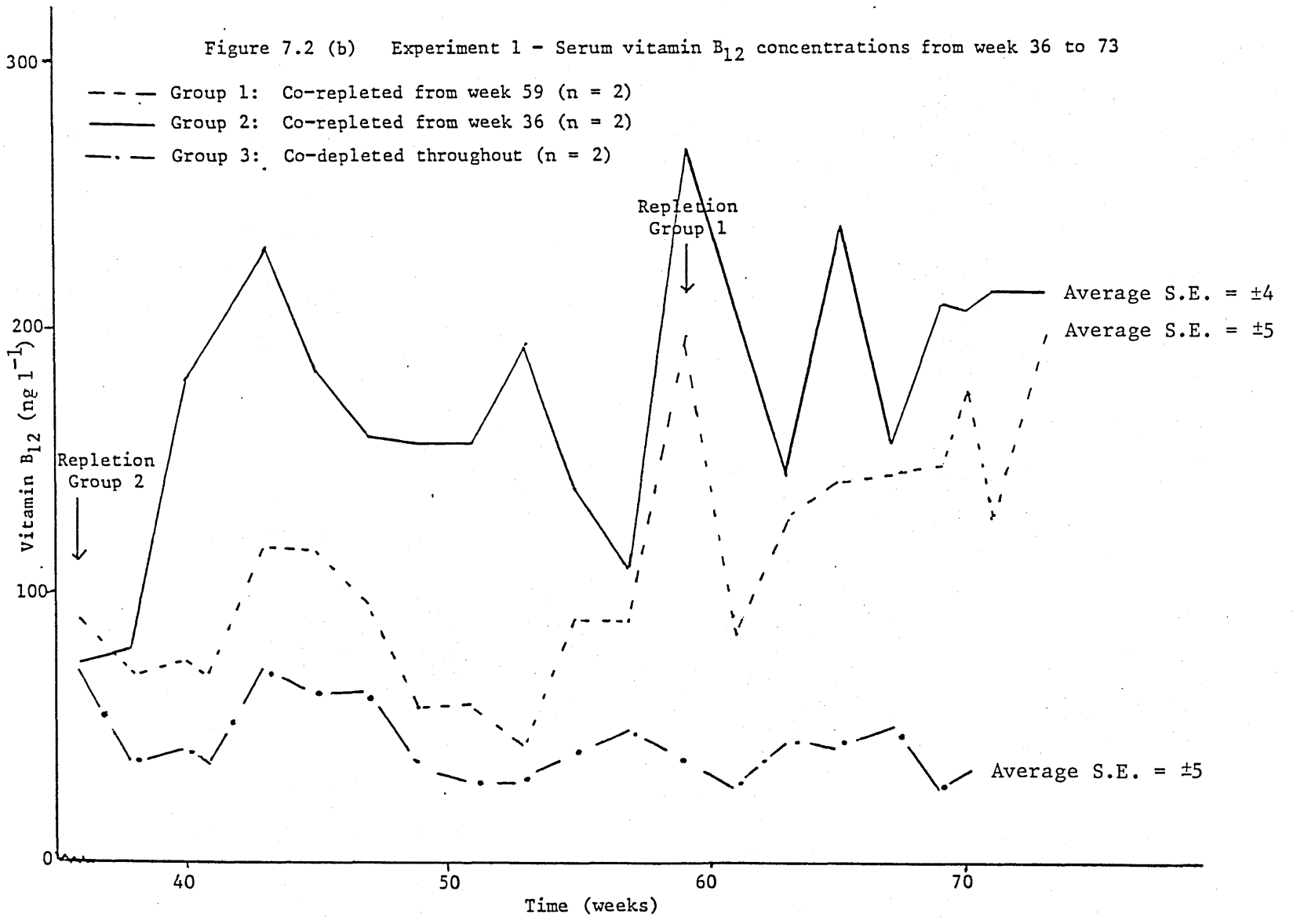


Figure 7.3 (a) Experiment 1 - Mean serum methyl-malonic acid to week 36 (n = 6)

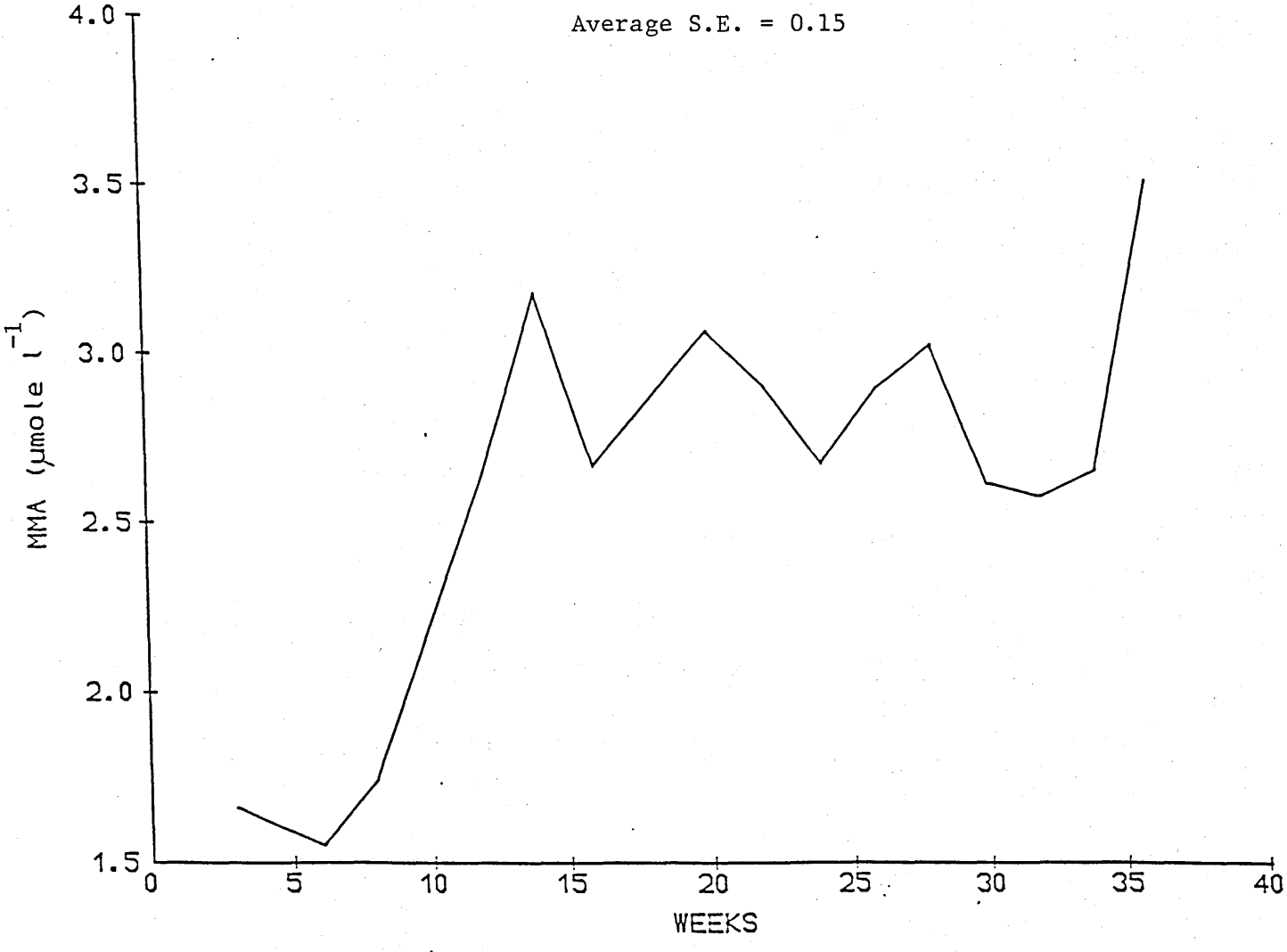


Figure 7.3 (b) Experiment 1 - Serum methyl-malonic acid from week 36 to 73

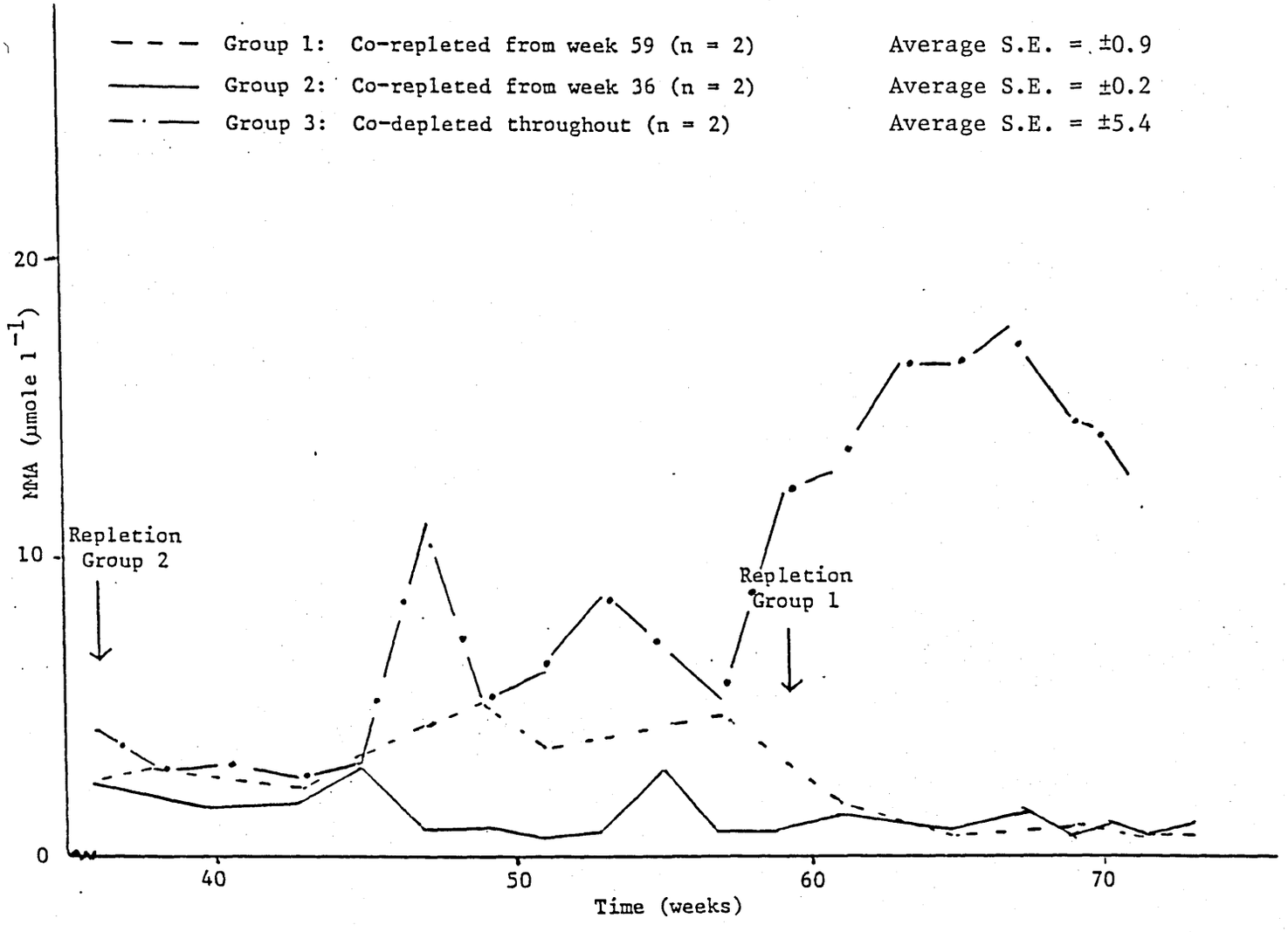


Figure 7.4 (a) Experiment 1 - Mean Neutrophil Function Test to week 36 (n = 6)

Average S.E. = ±1.4

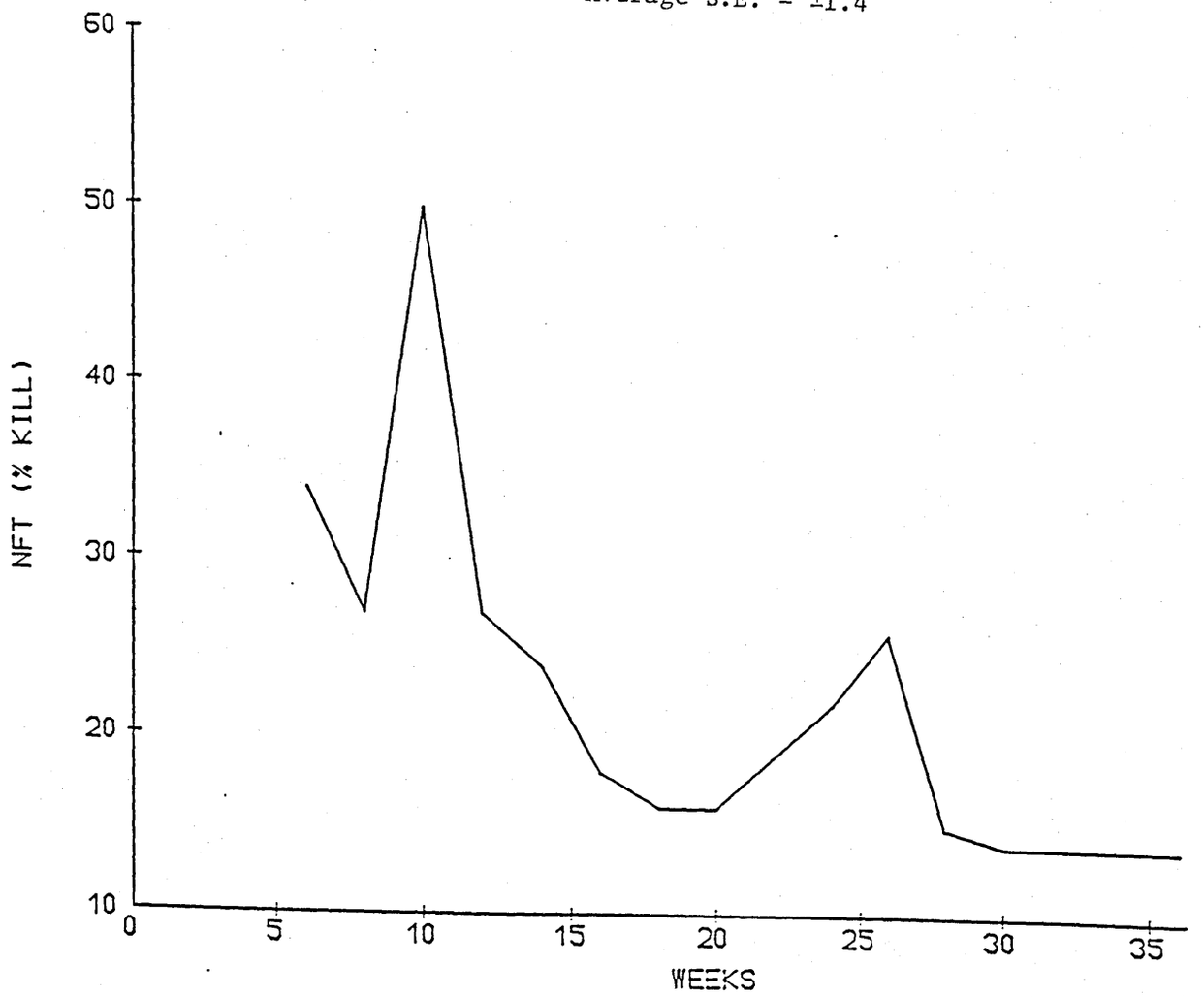
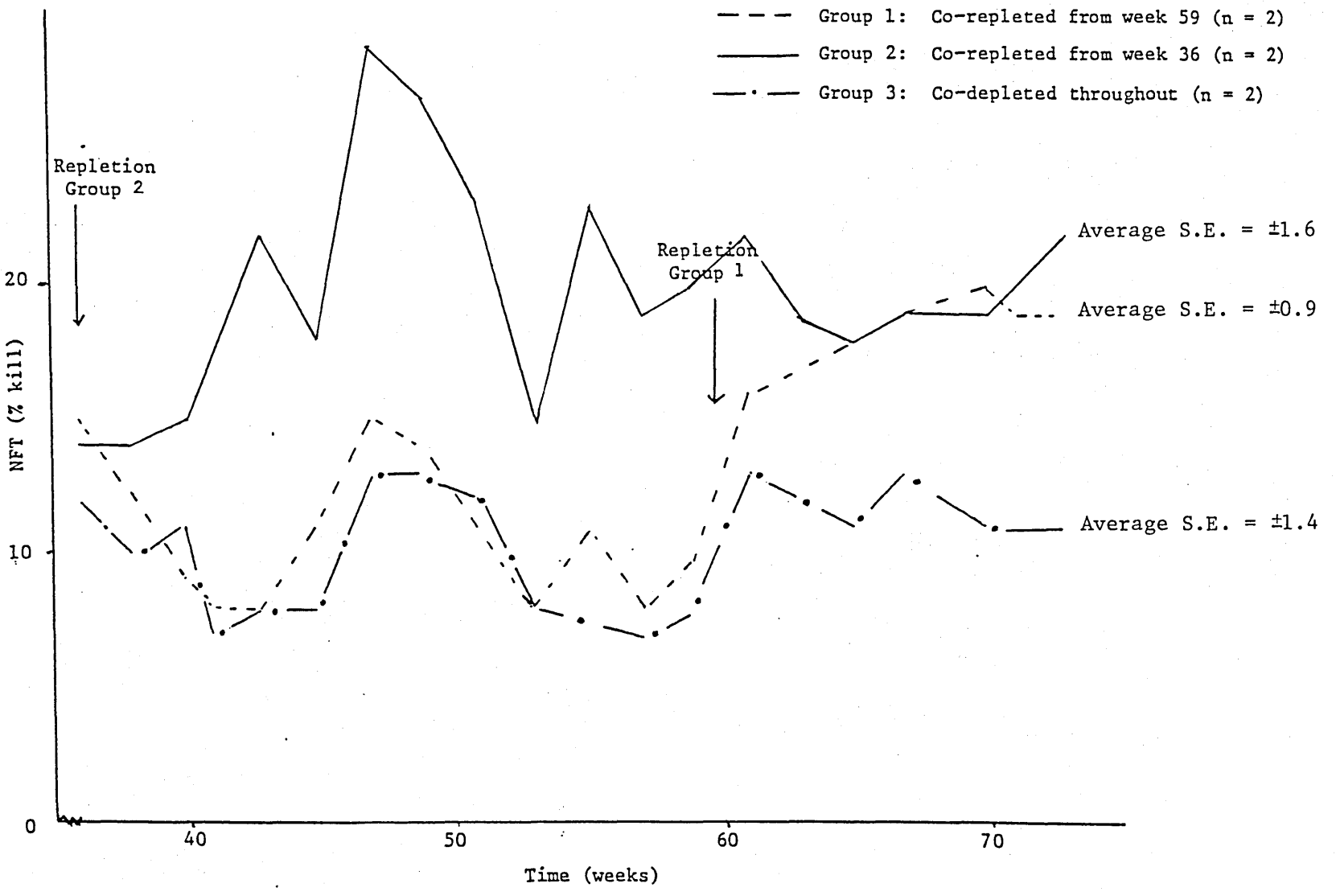


Figure 7.4 (b) Experiment 1 - Neutrophil Function Test from week 36 to 73



concentrations remained at <1.0 μmole l^{-1} from week 65 onwards. All the Co treatment methods produced an improvement in the % kill values obtained for the neutrophil function test by 4 to 6 weeks after treatment commenced (Fig. 7.4 (b)).

Despite very low serum vitamin B_{12} concentrations of <50 ng l^{-1} and elevated MMA values (approximately 15 μmole l^{-1}), the two cattle remaining on the deficient diet only showed a decline in liveweight performance, compared to the repleted animals, after 67 weeks (Fig. 7.1 (b)). When a 4000 μg vitamin B_{12} injection was administered to these animals at week 71, serum vitamin B_{12} concentrations rose to a mean of 163 ng l^{-1}, and MMA values declined to only 0.62 μmole l^{-1}, a fortnight after treatment, but had not yet affected the ability of the neutrophils to kill the yeast Candida albicans.

7.3.2 Experiment 2 - A comparison of immunocompetence and Co status of Co supplemented and depleted cattle

Co supplementation, although producing higher serum vitamin B_{12} values, lower MMA concentrations and a greater % kill of the yeast Candida albicans by isolated neutrophils, resulted in no improvement in liveweight performance over depleted animals until week 41 (Fig. 7.5). However, after 43 weeks on experiment, the Co-supplemented animals were significantly ($P < 0.05$) heavier than the untreated cattle.

Initially, serum vitamin B_{12} concentrations (by both assay methods) were around 200 ng l^{-1}, but increased to >300 ng l^{-1} by week 20 for Co supplemented cattle and remained at <100 ng l^{-1} from week 10 onwards for depleted animals (Fig. 7.6). By week 35, very low vitamin B_{12} levels for the unsupplemented animals of around 50 ng l^{-1} were recorded irrespective of assay procedure. In general,

Figure 7.5 Experiment 2 - Mean liveweights

Average S.E. = ±3.5

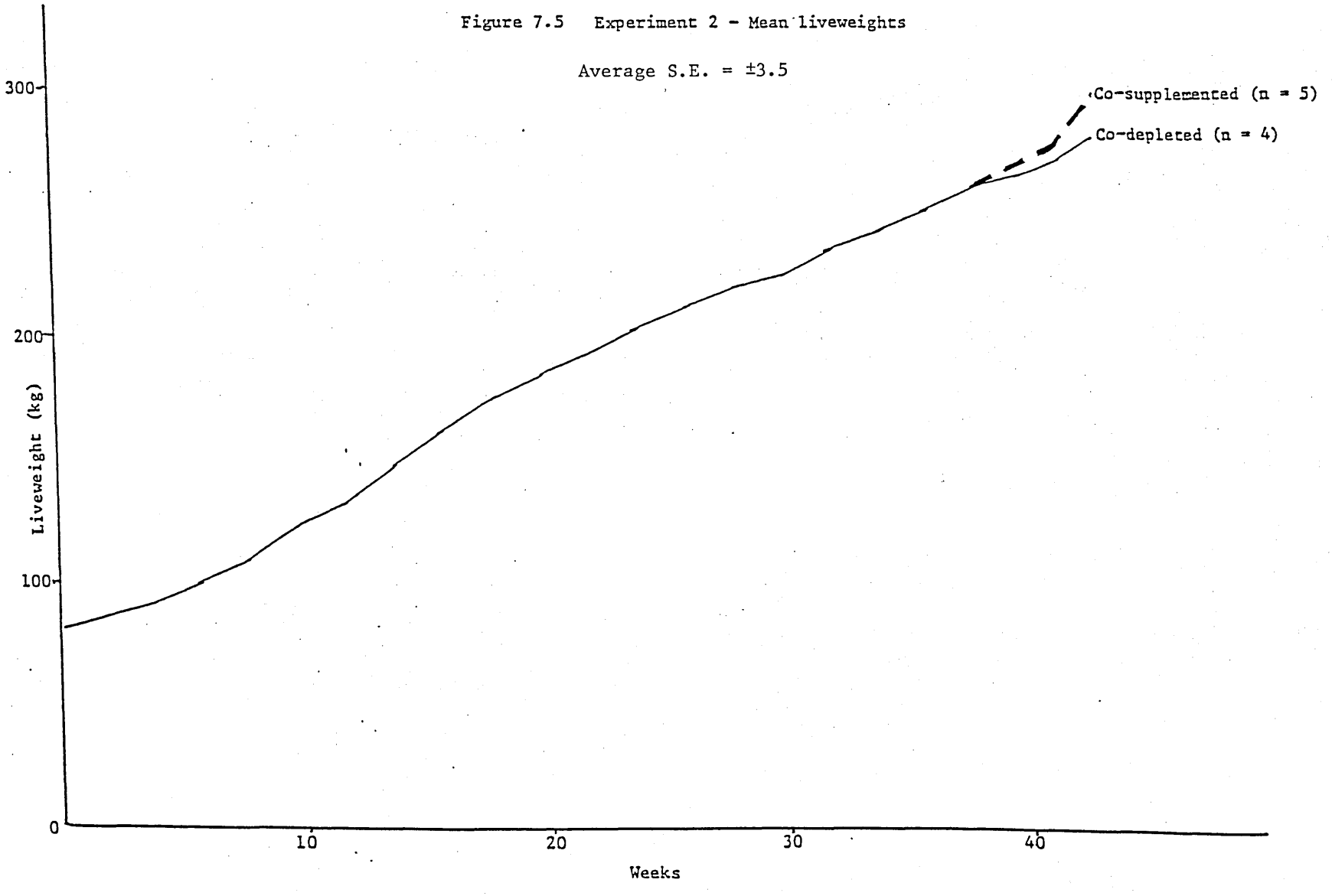


Figure 7.6 Experiment 2 - Mean serum vitamin B_{12}

(a) Microbiological determination

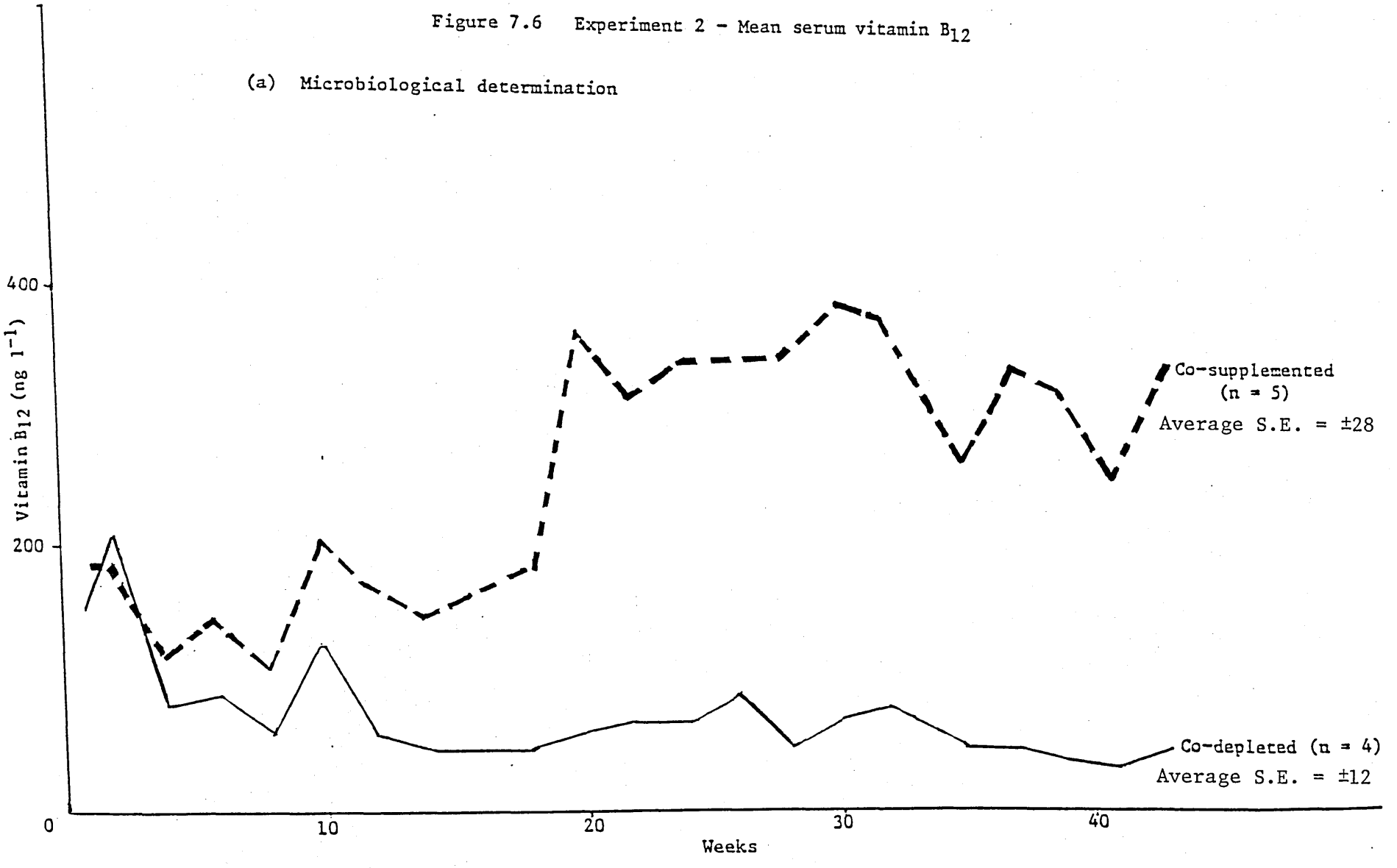
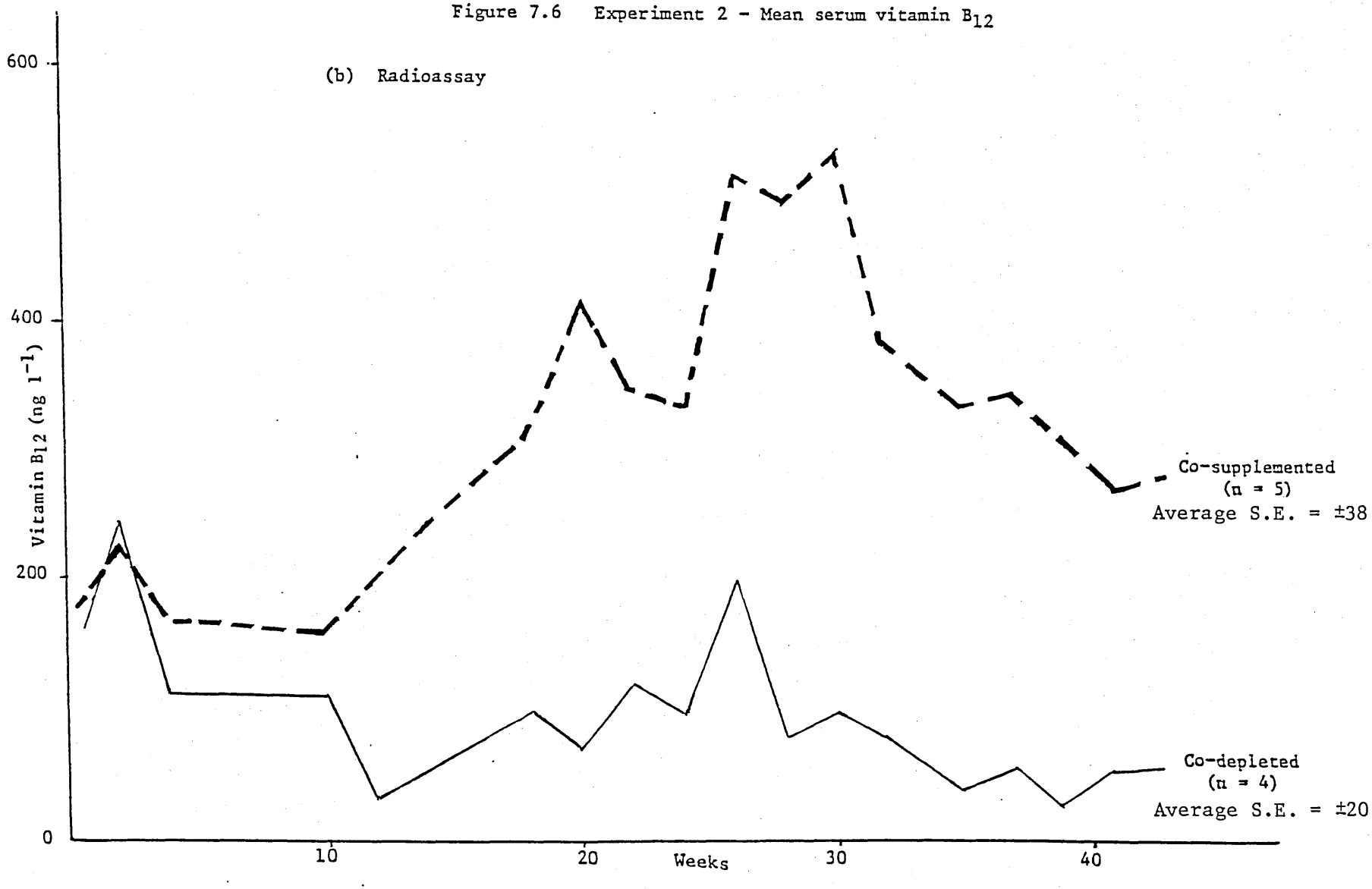


Figure 7.6 Experiment 2 - Mean serum vitamin B_{12}



vitamin B_{12} levels as determined by radio-assay were higher than those obtained by the microbiological method, with this being more evident for the Co-supplemented animals. This is illustrated by the following regression equations:

(y = microbiologically determined vitamin B_{12})

Deficient cattle:

$y = 0.65$ (radio-assay) vitamin $B_{12} + 13.9$ $\quad SD_y = 25.47$

$r = 0.81$ $\quad (P < 0.001)$

Co-supplemented cattle:

$y = 0.65$ (radio-assay) vitamin $B_{12} + 64.7$ $\quad SD_y = 50.04$

$r = 0.83$ $\quad (P < 0.001)$

Although both methods of vitamin B_{12} determination showed highly significant ($P < 0.001$) influences of treatment at week 20, the first appearance of significant differences was seen at week 10 for the microbiologically determined vitamin B_{12} and week 12 for the radio-assay technique.

On the other hand, significant differences in MMA concentrations did not appear until week 18, some eight weeks after vitamin B_{12} (Fig. 7.7). This was probably due to the large animal variation especially within the depleted group which is of more interest for diagnostic purposes. For example, at week 41, two depleted animals with similar serum vitamin B_{12} concentrations produced MMA values of 7.80 and 2.36 μmole l^{-1}.

The Co-supplemented animals maintained the initial % kill values obtained for the neutrophil function test throughout the trial, while those cattle on the depletion diet recorded a drop in immune status to give a mean of only 10% kill by week 43 (Fig. 7.8). From week 6, supplemented animals gave significantly ($P < 0.01$) higher % kill values.

Figure 7.7 Experiment 2 - Mean serum methyl-malonic acid concentrations

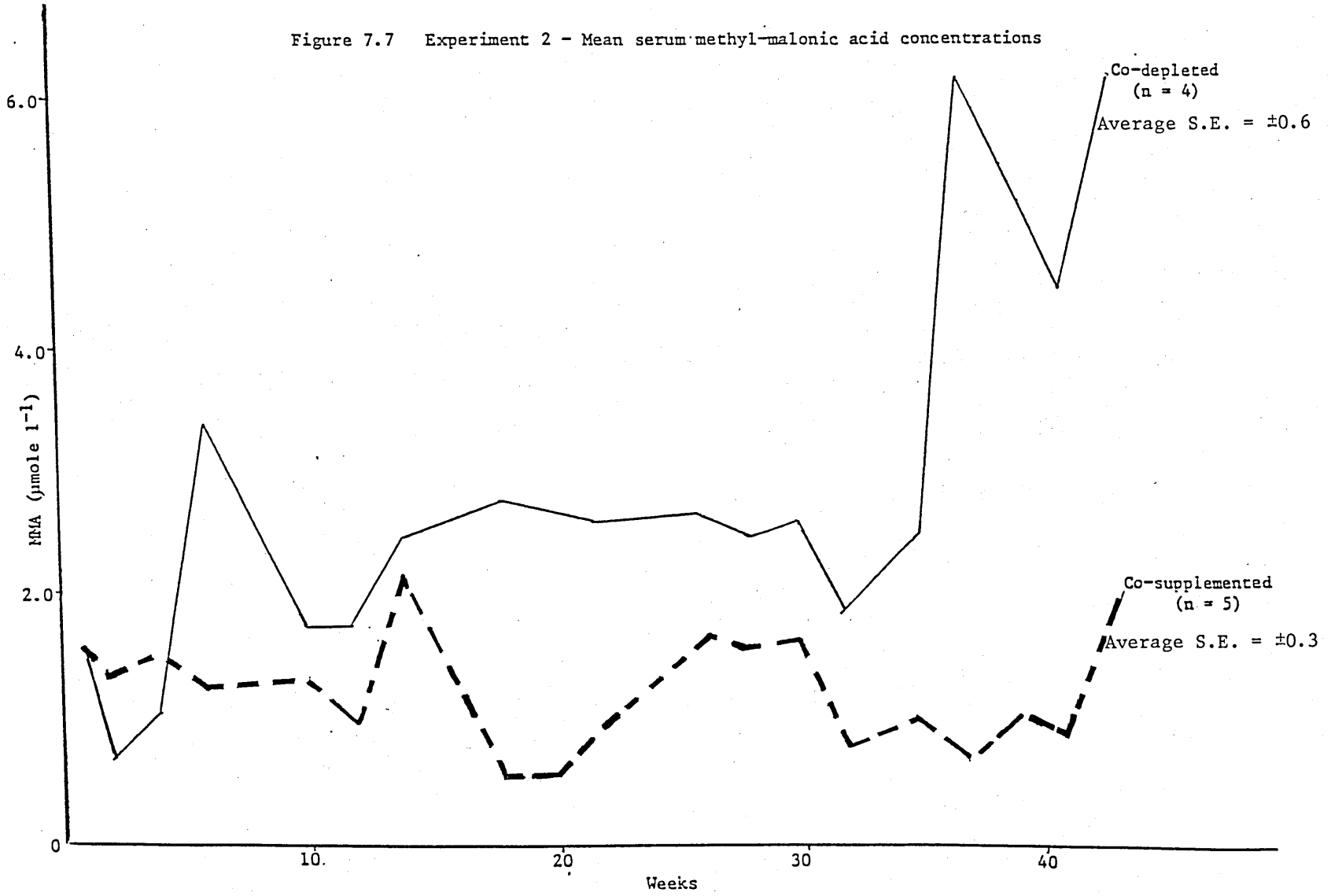
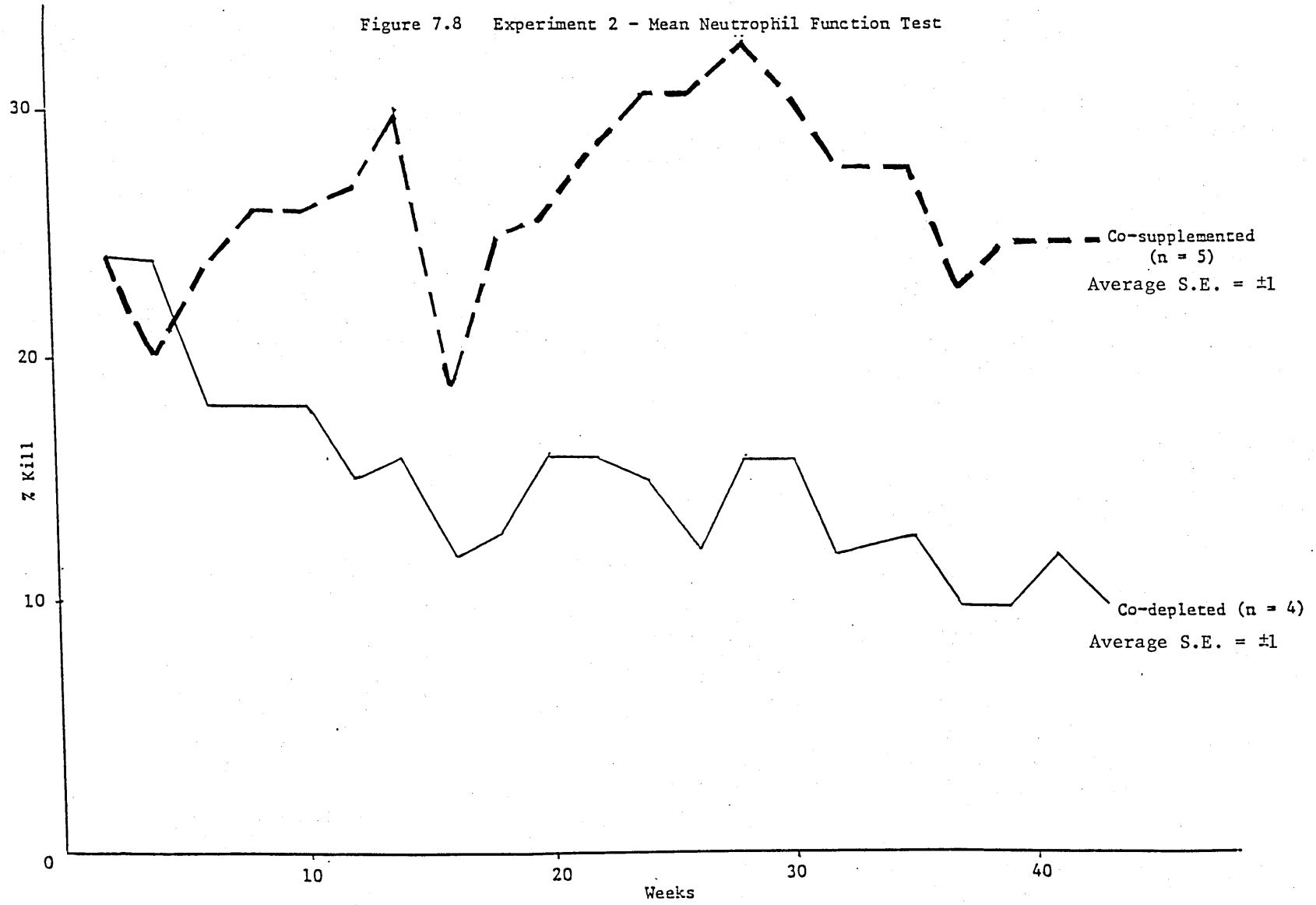


Figure 7.8 Experiment 2 - Mean Neutrophil Function Test



7.3.3 Experiment 3 - Effects of Co status on the degree of severity of Ostertagia ostertagi infection

Both Co status and the administration of gastro-intestinal parasites influenced the liveweight performance and appearance of the cattle. For both control and infected groups, Co-supplementation led to improved liveweights (Fig. 7.9) and coat colour. An initial mean difference of 10 kg between supplemented and depleted control animals was increased to 40 kg by week 9. This effect, however, was reduced when parasites were given. Parasitic infection led to a weight loss 4 weeks after initial administration of the larvae. Both the supplemented and depleted animals produced a similar pattern of weight loss until week 6 but thereafter, deficient animals lost more weight. At week 6, clinical symptoms of parasitic infection appeared as severe scouring in one supplemented and two deficient animals. One of these deficient animals lost 25 kg between weeks 6 and 8 and appeared weak and listless standing away from the rest. By week 7 all the infected animals were refusing their feed and between weeks 7 and 8 a liveweight loss of 6 kg in the depleted infected group was recorded compared with a 2 kg weight loss for those cattle receiving Co supplementation. After the administration of the anthelmintic at week 8, loss of weight continued for a further three days, when all animals began to gain weight and eat increasing amounts of feed to give an average gain of 10 kg after one week (Table 7.2).

Serum vitamin B_{12} concentrations, irrespective of assay procedure, were unaffected by parasitic infection (Fig. 7.10), with Co-supplementation producing values of around 300 ng l^{-1} compared with 50 ng l^{-1} for unsupplemented animals. Within the Co-

Figure 7.9 Experiment 3 - Influence of Ostertagia ostertagi infection on liveweight

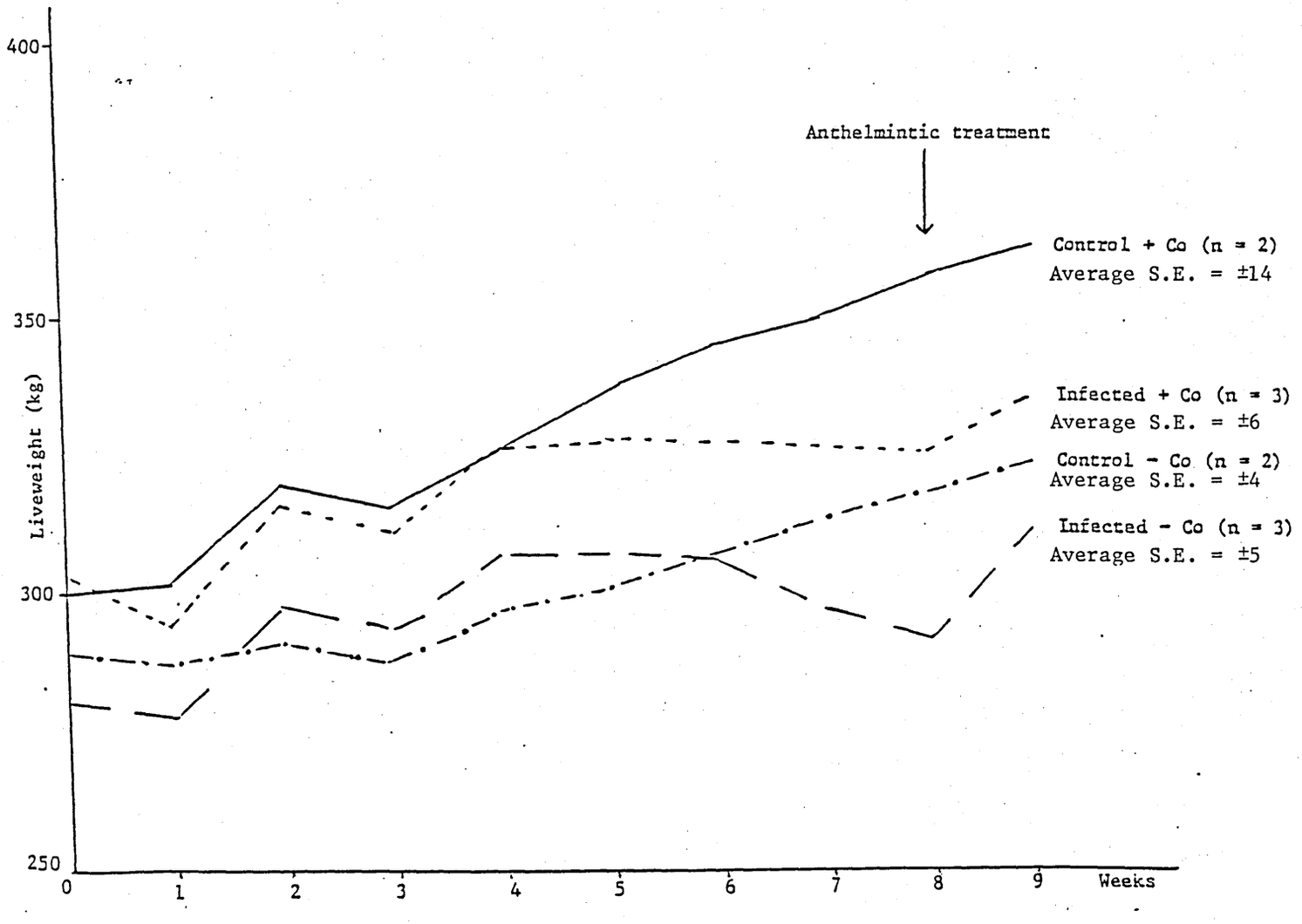


Figure 7.10 Experiment 3 - Influence of Ostertagia ostertagi infection on serum vitamin B_{12}

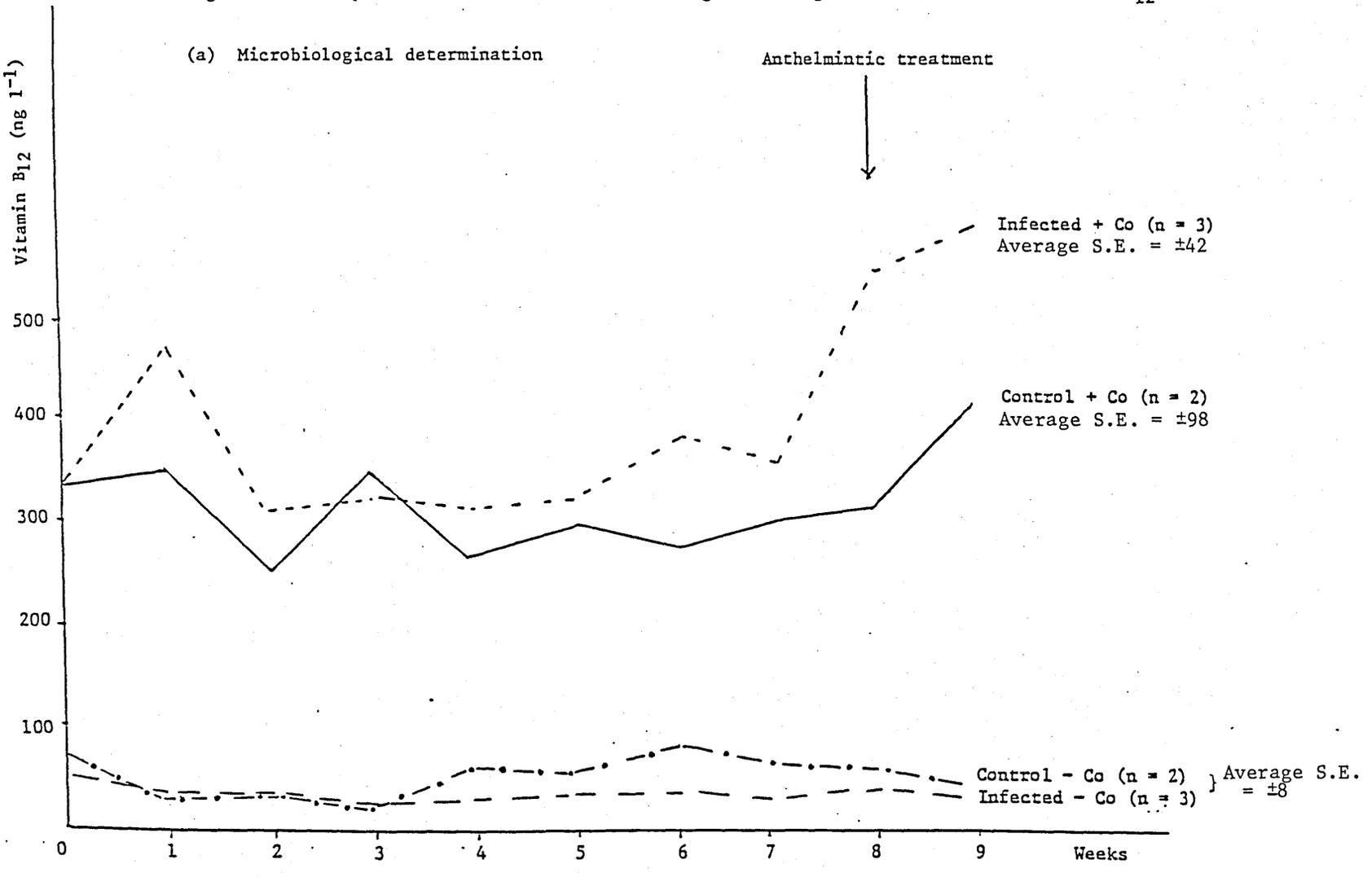
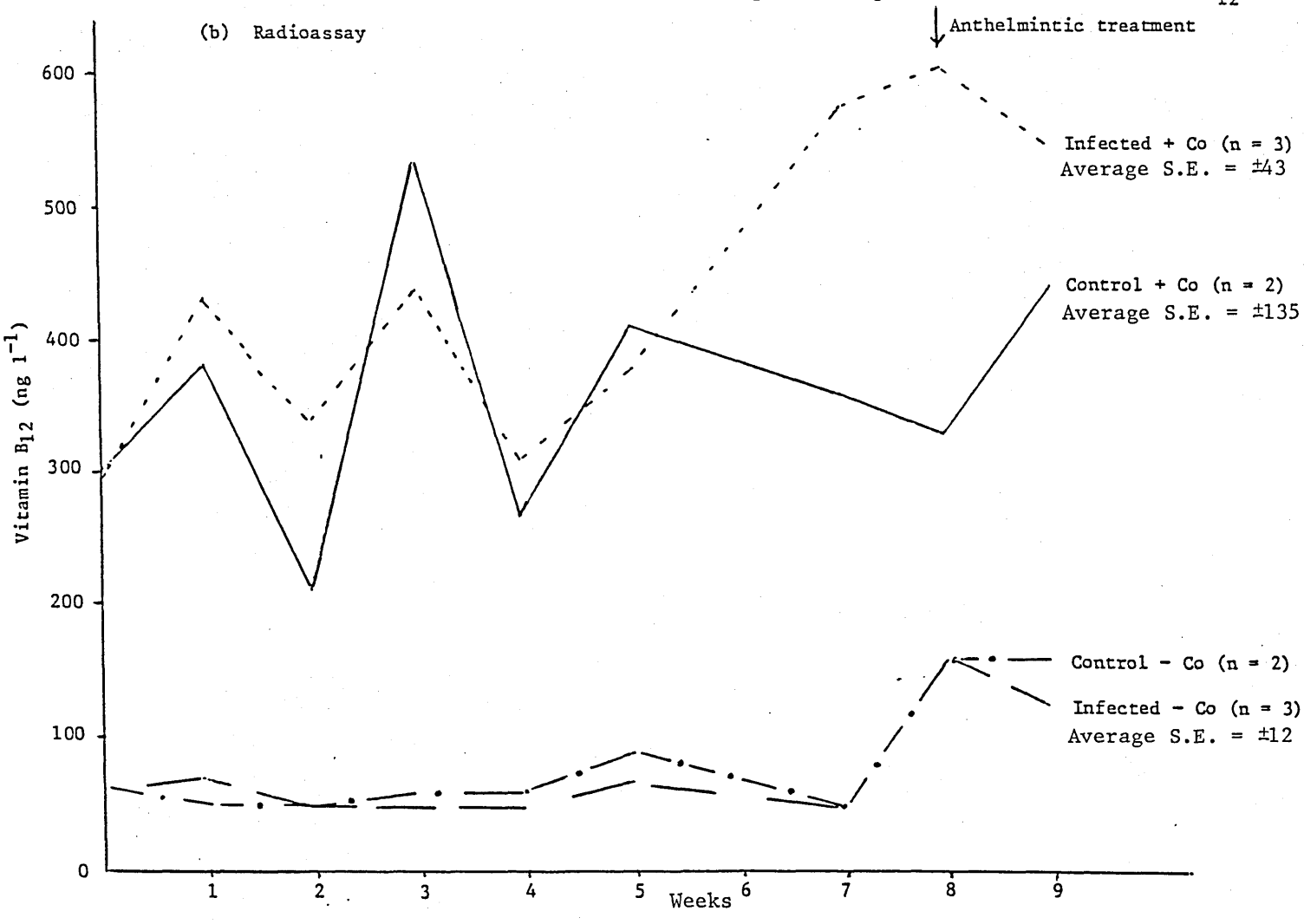


Figure 7.10 Experiment 3 - Influence of Ostertagia ostertagi infection on serum vitamin B_{12}



supplemented groups, larger variations were seen as a result of the fortnightly vitamin B_{12} injection having its greatest effect after one week. After administration of the Co-containing anthelmintic, at week 8, serum vitamin B_{12} levels were enhanced but for only a short period (Table 7.2). For the depleted group, serum vitamin B_{12} fell within 24 hours to pre-treatment levels, after a small boost in Co status after one hour. However, the supplemented animals maintained elevated values for 7 days but this was probably due more to the vitamin B_{12} injection given initially than from the Co-anthelmintic. As seen in experiment 2, vitamin B_{12} measured by radio-assay gave higher values than microbiological determinations, but the correlation between the two methods was poor:

Deficient cattle:

$y = 0.51$ (radio-assay) vitamin $B_{12} + 15.0$ $\quad SD_y = 14.33$

$r = 0.22$ (non-significant)

Supplemented cattle:

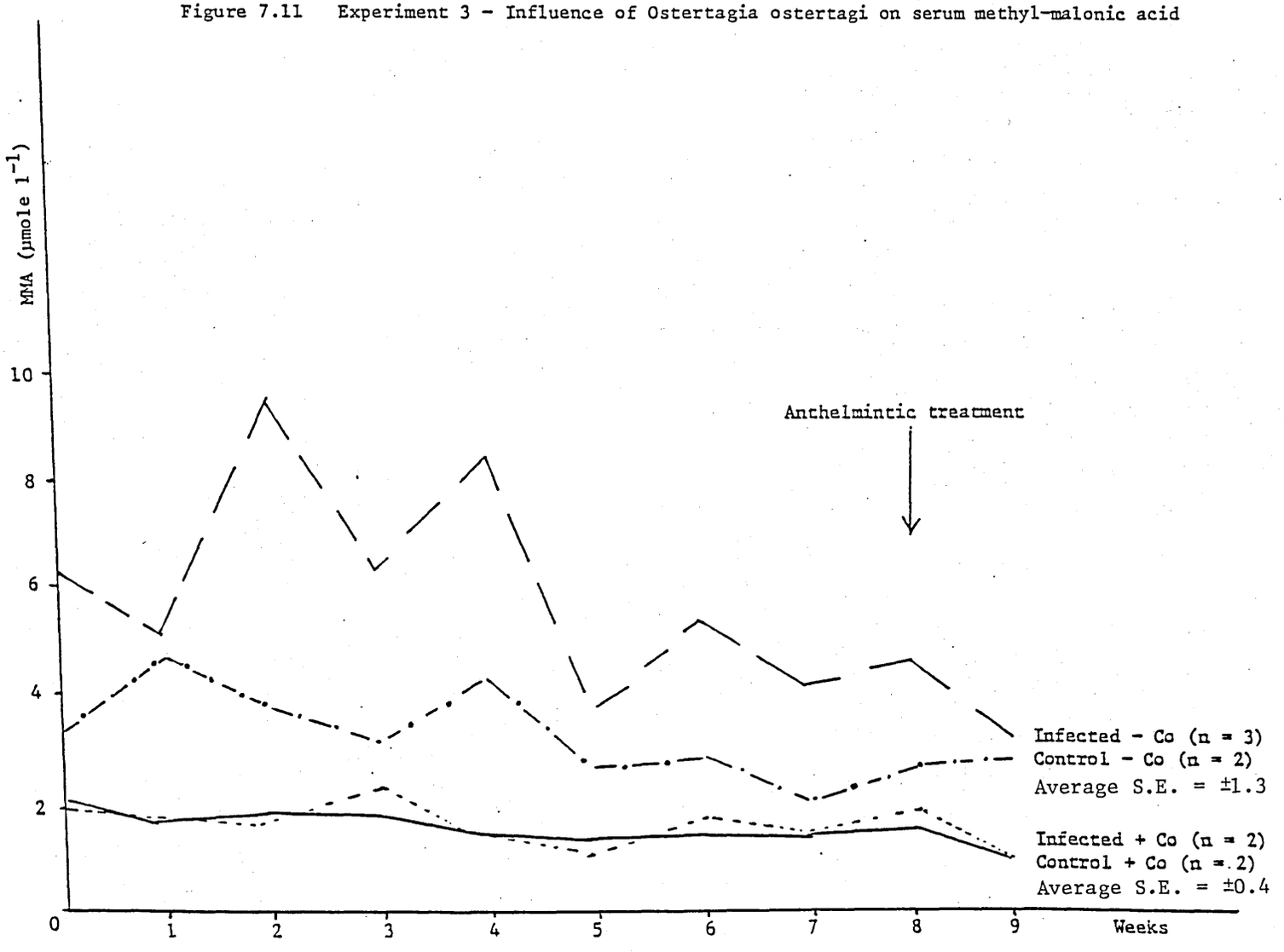
$y = 0.15$ (radio-assay) vitamin $B_{12} + 263$ $\quad SD_y = 57.18$

$r = 0.01$ (non-significant)

where y = vitamin B_{12} (microbiological assay)

As with serum vitamin B_{12}, MMA concentrations were not significantly affected by parasitism (Fig. 7.11). Both Co-supplemented groups maintained MMA values of around 1.50 μmole l^{-1}. In contrast, for the depleted animals, parasitic infection resulted in MMA concentrations of around 5.00 μmole l^{-1} compared with approximately 2.50 μmole l^{-1} for uninfected animals. As in Experiments 1 and 2, large animal variation occurred. For example, two deficient cattle of similar vitamin B_{12} status produced MMA values of 1.79 and 5.60 μmole l^{-1} and while most of the Co supplemented animals gave MMA concentrations in the range 0.5-1.5 μmole l^{-1},

Figure 7.11 Experiment 3 - Influence of Ostertagia ostertagi on serum methyl-malonic acid



one Co-treated animal maintained MMA concentrations of 2.00 μmole l^{-1} throughout the trial. Furthermore, when severe parasitic infection was established in one Co-deficient animal, MMA concentrations fell to 1.00-2.00 μmole l^{-1} as the animal refused feed, but increased to 3.50 μmole l^{-1} when appetite was restored after anthelmintic treatment. In general, administration of the Co-containing anthelmintic at week 8 had little effect on MMA values (Table 7.2). For Co-supplemented animals, MMA concentrations fell to around 1.00 μmole l^{-1} one day after treatment, while depleted animals maintained values of around 4.00-5.00 μmole l^{-1} for 5 days after which levels fell to approximately 3.5 μmole l^{-1}.

The administration of parasites had no effect on neutrophil function (Fig. 7.12) with Co-supplementation maintaining significantly ($P < 0.001$) higher % kill values than unsupplemented animals.

Gastro-intestinal parasite infection led to an increase in serum pepsinogen levels with control animals producing values within the normal range throughout the trial (Fig. 7.13). Co status had no effect on the concentration of pepsinogen, with both infected groups producing increased values after 3 weeks and the levels continuing to rise throughout the trial to >3.00 i.u. l^{-1} by week 8. Following anthelmintic treatment, pepsinogen concentrations showed a slight fall after 3 days when appetite improved (Table 7.2). In addition, 26 days after treatment, pepsinogen values of 1.19 (±0.11) and 0.98 (±0.19) i.u. l^{-1} for Co-supplemented and depleted cattle respectively were recorded, while at day 35 both groups had pepsinogen concentrations similar to those of the control uninfected animals (0.66 ± 0.15 i.u. l^{-1}).

Figure 7.12 Experiment 3 - Influence of Ostertagia ostertagi infection on the neutrophil function test

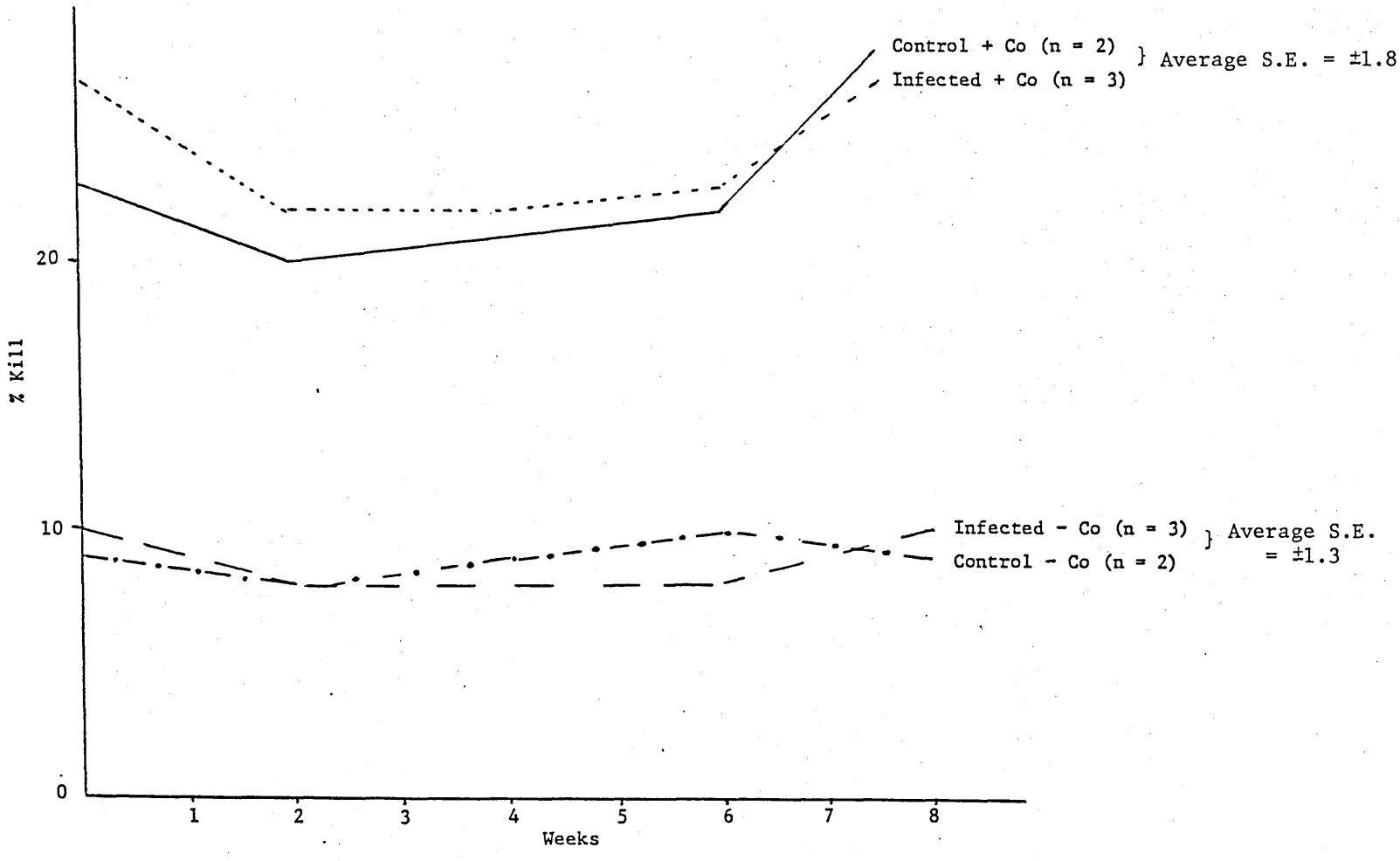
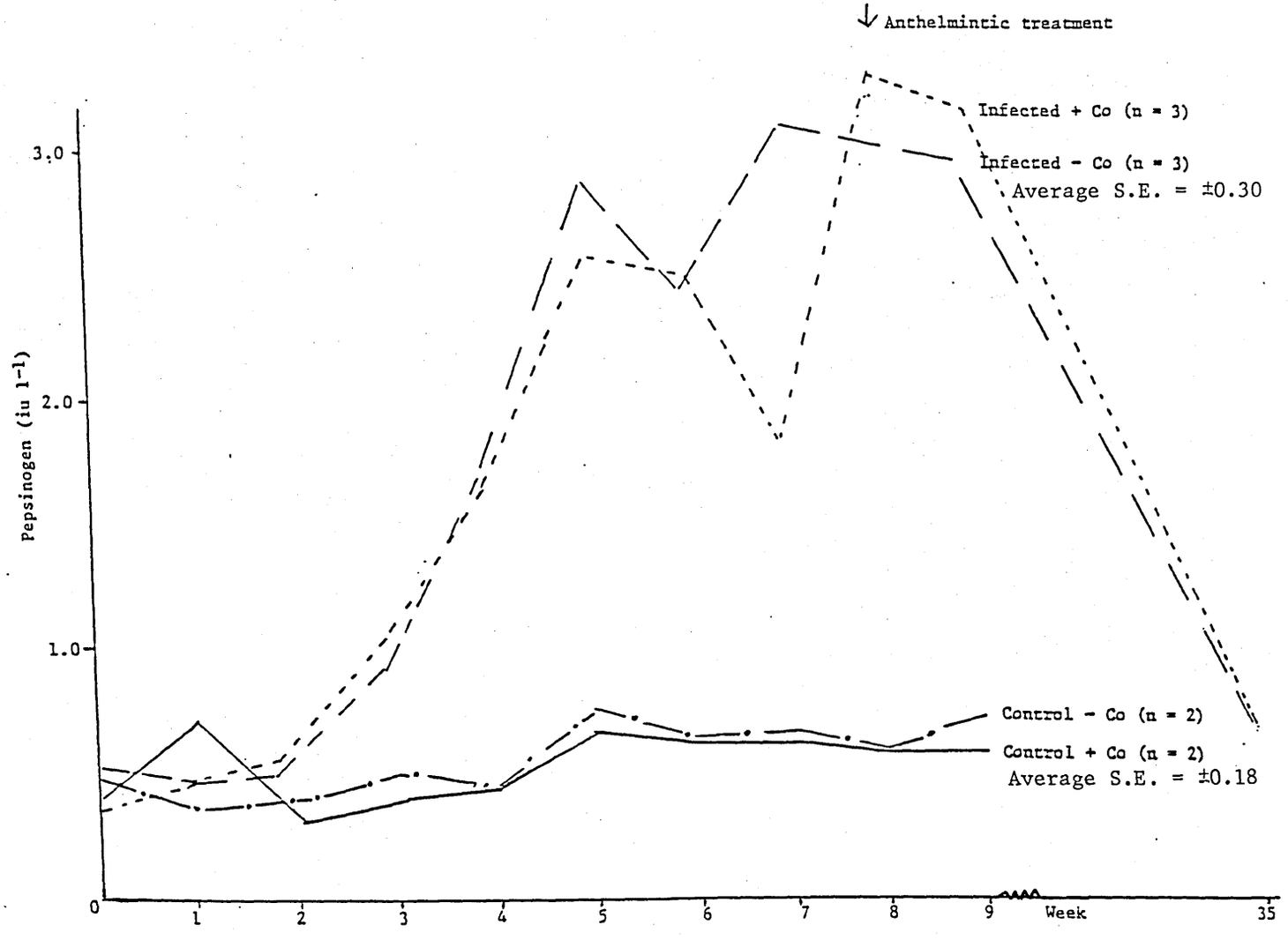


Figure 7.13 Experiment 3 - Influence of Ostertagia ostertagi on serum pepsinogen



Ostertagia ostertagi infection led to elevated gastrin concentrations in a similar way to its effect on serum pepsinogen, while control animals maintained values within the normal range of 0-100 pg ml^{-1} (Fig. 7.14). Co status had no significant influence on gastrin. Both infected groups showed slightly increased values after 2 weeks but levels did not rise to >100 pg ml^{-1} until week 4. Thereafter very high values of >500 pg ml^{-1} were recorded from week 6 onwards. On administration of the anthelmintic at week 8, the Co-supplemented group showed a fall in gastrin concentrations after 2 days and this continued until the end of the trial (Table 7.2). On the other hand, the depleted group produced a decline in gastrin values after 3 days but 2 days later the levels increased (Table 7.2). For both groups, gastrin concentrations had fallen to around 400 pg ml^{-1} 7 days after treatment.

All the animals, irrespective of treatment, showed high inorganic phosphorus values of >2.5 mmol l^{-1}, <2.5 mmol l^{-1} being the normal values (Fig. 7.15). The infected animals produced lower concentrations 6 weeks after infection but these values were still above normal concentrations. One week after anthelmintic treatment, initial values were re-established (Table 7.2).

Co status had no effect on the length of the prepatent period or on worm egg counts. For both groups patency was reached around 21-25 days. At no time throughout the trial were high worm egg counts seen (Fig. 7.16) and 5 days after anthelmintic treatment no worm eggs were found in the dung (Table 7.2).

7.4/

Figure 7.14 Experiment 3 - Influence of Ostertagia ostertagi infection on serum gastrin

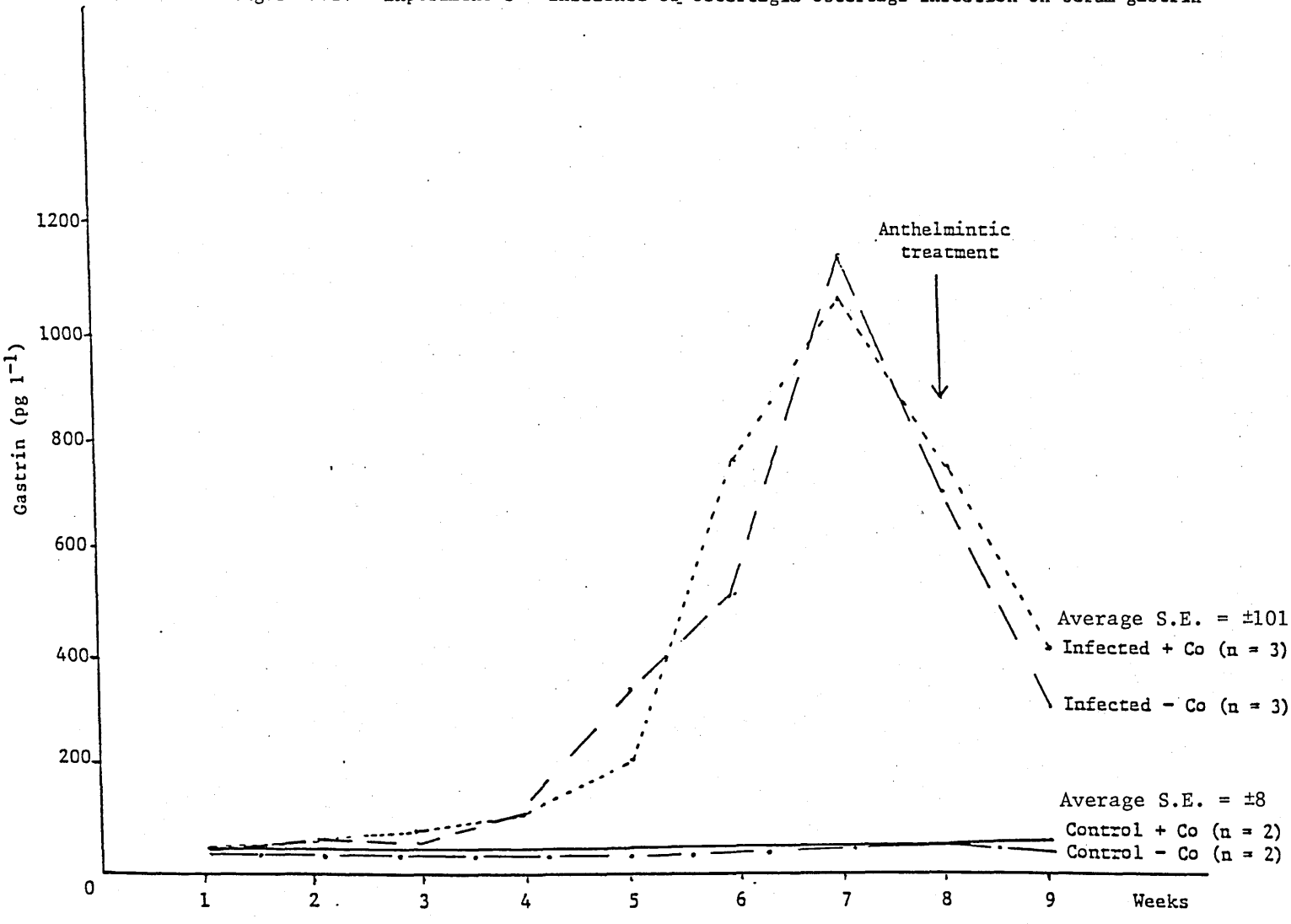


Figure 7.15 Experiment 3 - Influence of Ostertagia ostertagi infection on serum inorganic phosphorus

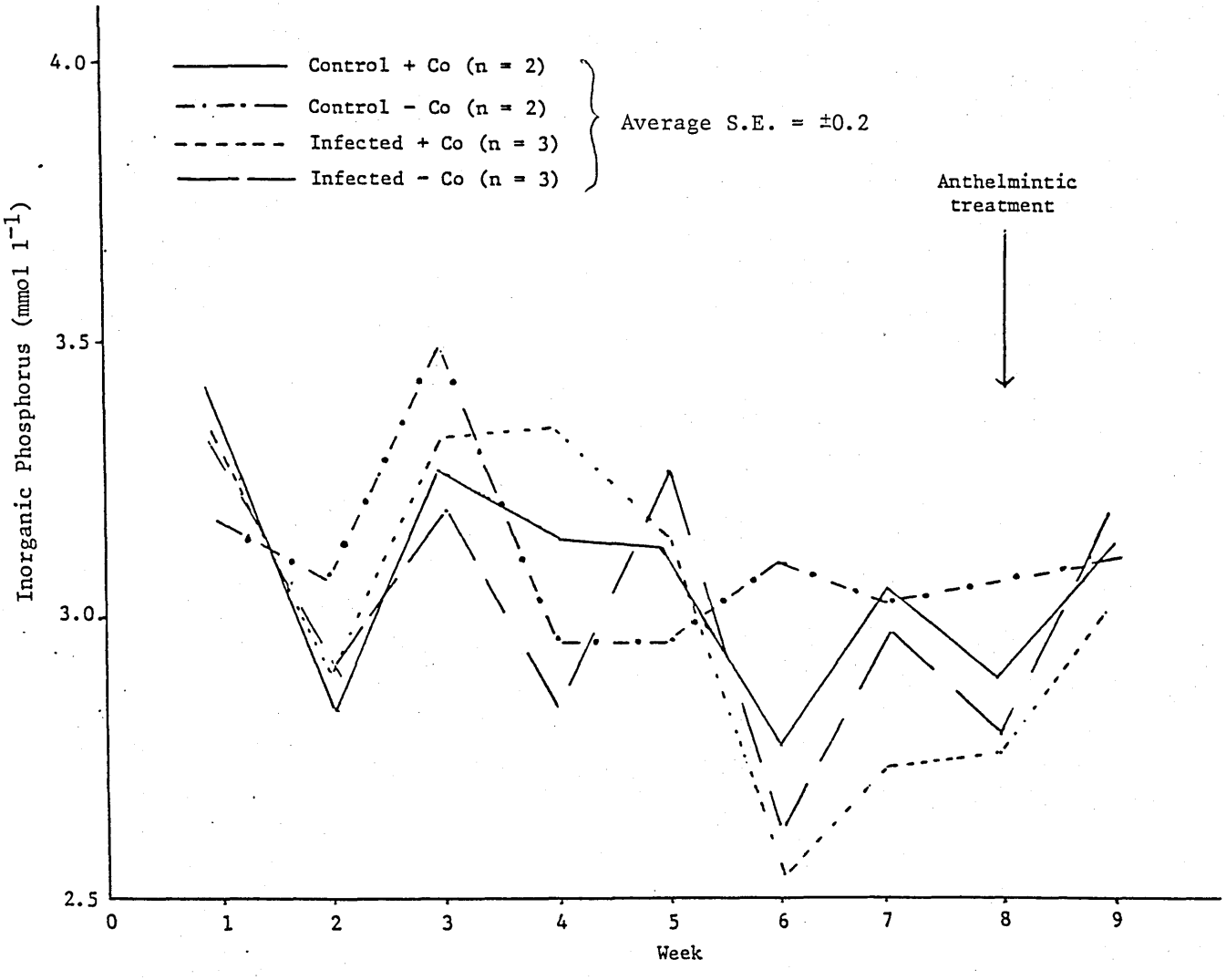


Figure 7.16 Influence of Ostertagia ostertagi infection on faecal worm egg counts

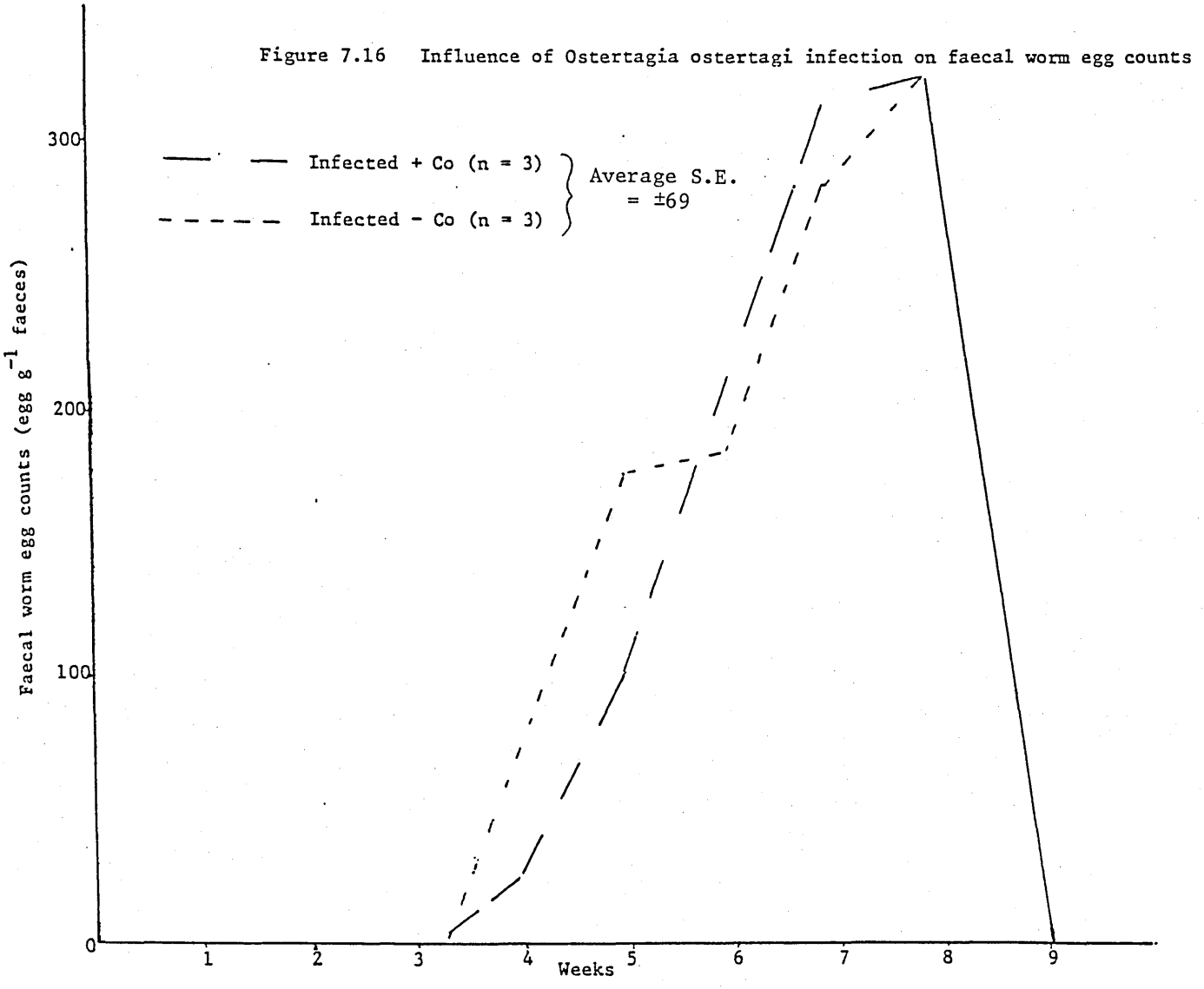


Table 7.2 Mean results of parameters measured from infected animals after anthelmintic treatment

	Co supplemented (n=3)								Depleted (n=3)							
	LWT	Vit B_{12}		MMA	Peps	Gastrin	P	WEC	LWT	Vit B_{12}		MMA	Peps	Gastrin	P	WEC
		Micro	RA							Micro	RA					
0 hr	316	555	610	1.9	3.33	787	2.76	333	292	63	160	4.6	3.10	718	2.79	333
1 hr	-	>1250	-	1.9	3.53	857	-	-	-	153	-	5.0	3.39	680	-	-
3 hrs	-	>1250	-	1.7	3.50	800	-	-	-	113	-	5.7	3.47	605	-	-
5 hrs	-	>1250	-	0.9	3.48	783	-	-	-	78	-	5.6	3.28	695	-	-
7 hrs	-	>1250	-	1.2	3.46	853	-	-	-	67	-	4.2	3.34	798	-	-
1 day	314	>1250	-	0.7	3.20	897	-	-	293	53	-	3.9	2.89	825	-	-
2 days	313	1133	-	0.9	2.23	750	-	-	293	50	-	3.8	3.03	848	-	-
3 days	321	903	-	1.4	3.09	633	-	-	299	43	-	6.0	2.79	668	-	-
5 days	322	725	-	1.3	2.92	567	-	0	297	55	-	3.6	2.64	880	-	0
7 days	326	598	550	1.1	3.20	423	3.02	-	302	43	132	3.6	3.02	312	3.20	-
Av S.E. (±)	12	117	33	0.5	0.23	151	0.15	-	10	14	15	2.1	0.6	343	0.15	-

Key: LWT = liveweight (kg)
Vit B12 = vitamin B12 (ng l^{-1})
[RA = radio-assay]
micro = microbiological
MMA = methyl-malonic acid (µmoles l^{-1})
Peps = plasma pepsinogen (i ul^{-1})
Gastrin = plasma gastrin (pg ml^{-1})
P = inorganic phosphorus (m moles l^{-1})
WEC = worm egg counts (eggs g^{-1} faeces)

7.4 DISCUSSION

7.4.1 Experiments 1 and 2

In both Experiments 1 and 2, a low Co dietary intake produced no effect on liveweight performance until weeks 67 and 41 respectively and at no time was a weight loss recorded. Other workers (MacPherson et al, 1983; MacPherson, 1982) did obtain a weight loss in steers 15-24 weeks after the introduction of a Co-deficient diet. In contrast, other findings have tended to agree with those given here with no effect on liveweight production seen despite very low serum vitamin B_{12} values even after 40-45 weeks on a Co-deficient diet (MacPherson, 1981, 1982; Wright et al, 1982). In order to explain these contrasting results MacPherson (1982) suggested that "some form of stress may be necessary to precipitate the clinical Co deficiency syndrome" such as subjection to wet and cold outdoor conditions. Further, during Experiments 1 and 2 no effect on appetite (the classic symptom of Co deficiency (Underwood, 1978)) was recorded despite producing serum vitamin B_{12} concentrations of <50 ng l^{-1}. However, in both these experiments, during the latter stages when prolonged inadequate dietary Co is likely to cause a loss of appetite, all the animals were in one large pen. Under such circumstances, any reduction in appetite by the depleted animals may have been compensated by an increased intake by Co supplemented animals.

Whereas liveweight was not influenced by Co status until the latter stages of Experiments 1 and 2, the degree of immunity as measured by the neutrophil function test was affected within a few weeks of feeding a Co-deficient diet. In a similar manner, MacPherson et al (1987) obtained a

drop in % kill of the yeast Candida albicans by isolated neutrophils when serum vitamin B_{12} concentrations of calves declined from 200 ng l^{-1} to approximately 100-150 ng l^{-1} within 7 weeks. Using pregnant ewes, Fisher and MacPherson (1986) also demonstrated a drop in immunocompetence within 10 weeks of feeding a Co-deficient diet with a consequent reduction in the viability of their lambs. Thus, despite a low Co intake showing no immediate effect on liveweight performance, the reduced immune response will lead to increased vulnerability to infection and hence a drop in animal productivity. To date the reasons why Co has such an effect on immune response are unknown.

Any diagnostic measurement of Co status must predict not only severe clinical Co deficiency, where loss of weight or reduced liveweight gains are recorded, but also sub-clinical Co deficiency where immunity is impaired and susceptibility to infection increased. For both experiments 1 and 2 immunocompetence was reduced when serum vitamin B_{12} concentrations (by both assays) declined to around 100 ng l^{-1} and MMA concentrations increased to approximately 2 µmole l^{-1}. However, serum vitamin B_{12} was not a sensitive indicator of clinical Co deficiency, with very low serum vitamin B_{12} values (<50 ng l^{-1}) seen for around 25 weeks before any reduction in liveweight gain was recorded. On the other hand, MMA concentrations increased to a mean >4.00 µmole l^{-1} in Experiment 2, a few weeks before liveweight production was affected and, therefore, MMA may provide a better indication of when clinical symptoms of Co deficiency are likely to appear than does serum vitamin B_{12} measurement.

Serum vitamin B_{12} concentrations measured by both assay techniques showed good agreement, although the radio-assay method in general

gave higher values and this was particularly so for Co-supplemented cattle. This is in contrast to Taylor and Greer (1982, 1983), who, when they used ovine serum, found that the radio-assay estimation consistently gave lower values. These findings of Taylor and Greer were as expected since while the microbiological assay technique measures all forms of vitamin B_{12}, the radio-assay method has been developed to estimate only "true" vitamin B_{12} and, hence, should give the lower estimate of serum vitamin B_{12}. Further, Halpin et al (1984) found that whereas sheep serum contains very low concentrations of inactive vitamin B_{12} analogues, in cattle a substantial proportion of vitamin B_{12} is inactive, being particularly so when Co intake is high. Hence, it would be expected that for cattle the radio-assay determination should give very much lower vitamin B_{12} estimations than the microbiological assay. This, however, was not found here, and may be due to some compound present only in cattle serum binding to the intrinsic factor used in the radio-assay technique and giving falsely elevated results. Serum MMA measurement, on the other hand, will not be subject to such problems and will therefore provide a direct assessment of whether propionate metabolism has been impaired as a result of Co deficiency. Past workers (McMurray et al, 1985; Fisher and MacPherson, 1986; Rice et al, 1987) have only investigated serum MMA concentrations in sheep so that no published data are available on cattle. In addition, Rice (1986) suggested that MMA contents in cattle serum may be difficult to interpret. In this study, however, serum MMA concentrations did show some promise as a diagnostic tool. The values obtained were lower in general than those seen for sheep, with normal values tentatively suggested as being <2 μmole l^{-1}, sub-clinical 2-4 μmole l^{-1} and deficiency >4 μmole l^{-1}. However, as the number of animals involved was small, further work is

required to establish more exact criteria. As with serum vitamin B_{12} determinations, MMA concentrations in cattle are subject to large animal variations necessitating the sampling of a relatively large number of animals in a herd before an overall appraisal can be made.

As suggested by Wright et al (1982), the critical level of 200 ng l^{-1} serum vitamin B_{12} for bovines used by the Veterinary Investigational Service appears to be too high. Certainly, for housed cattle, vitamin B_{12} concentrations of <200 ng l^{-1} can exist with no adverse influence on animal performance. From this work it would appear that the critical concentrations may also be dependent on the assay technique used and different interpretive criteria are required for each procedure.

Co repletion in Experiment 1 resulted in improved serum vitamin B_{12} concentrations, lower MMA contents and higher % kill values in the neutrophil function test. Whereas the administration of a slow-release Co bolus or Co needle brought about an improvement in Co status within a few days, weekly oral drenching initially with 10 mg Co was inadequate to increase serum vitamin B_{12} concentrations and reduce MMA contents. Even after increasing the quantity of Co given in the dose and administering vitamin B_{12} injections, serum MMA concentrations did not decline until 11 weeks after treatment commenced, indicating propionate metabolism remained impaired for some time after Co treatment started. Hence, it would appear that a low steady supply of Co from a slow-release bolus or needle was a more effective method of restoring Co status than the more transient administration of Co in an oral drench.

7.4.2 Experiment 3

Similar to the findings of other workers (Downey, 1965, 1966; Andrews et al, 1970; MacPherson et al, 1987), the work presented here suggests that animals of low Co status are more affected by Ostertagia ostertagi infection. In the initial stages both infected groups showed a similar liveweight pattern but between weeks 7 and 8 a greater weight loss was recorded for the depleted group. Further, on an individual basis, the one animal showing the most severe symptoms of parasitism belonged to the unsupplemented group. While MacPherson et al (1987) found patency was delayed by around a week for Co-supplemented cattle, this work demonstrated that patency was unaffected by the administration of Co. Similarly, faecal worm egg counts between the two groups remained very similar despite MacPherson et al (1987) having obtained significantly ($P < 0.05$) greater faecal worm egg output in Co - deficient animals than those receiving supplementary Co. However, for adult cattle especially there is little relationship between worm egg counts and Ostertagia ostertagi infection, since very few eggs are produced making measurement difficult. In contrast, for younger cattle, as used by MacPherson et al (1987), faecal worm egg counts are more useful in diagnostic determinations (Mitchell, 1988). Since all the infected animals were housed in one pen, it cannot be determined whether Co status had any effect on the extent of loss of appetite at week 7. On anthelmintic treatment both infected groups showed an identical response with appetite increasing after 3 days.

Both serum gastrin and pepsinogen concentrations on infection followed similar patterns with both parameters showing increased values from week 3 onwards. Co status had no influence on either

the time between the commencement of infection and the first observation of the elevated concentrations or the actual values obtained. This is in contrast to MacPherson et al (1987), who obtained significantly greater pepsinogen contents for Co-sufficient as opposed to depleted animals.

In the past, blood diagnosis of Ostertagiasis has relied on the use of serum pepsinogen which becomes elevated when infection becomes established as a result of damage to abomasal walls causing pepsinogen to leak into the blood-stream (Porter, 1977). However, serum pepsinogen concentrations can increase due to other causes which damage the stomach lining and Fox et al (1987), Jacobs et al (1987), Fox et al (1988) have examined the use of the hormone, gastrin, as a potential diagnostic measure. In addition, Fox (1988) suggests that the rise in blood gastrin concentration may, in part, be responsible for the observed drop in appetite of clinically infected animals, and, hence, such a measurement should be a more reliable indicator of an impairment in the animals' metabolism than serum pepsinogen. Fox (1988) demonstrated that in the field, blood gastrin and pepsinogen responses to Ostertagia infection were similar. However, Fox et al (1988) found that the diet which was fed strongly influenced the gastrin response to Ostertagia with peak values almost doubling when hay as opposed to a largely concentrate diet was used. Indeed, in the present study the values obtained lay between those given by Fox et al (1988) when they used either a largely concentrate or a hay only diet. Fox et al (1987) also found as was reported here (Fig. 7.14) that gastrin concentrations declined before anthelmintic treatment. This may have been due to a loss of worms at this stage as suggested by Murray et al (1970). Such an effect was not observed

for serum pepsinogen. Hence, serum gastrin appears to offer some potential as a diagnostic tool with values in excess of 100 pg ml^{-1} indicative of some parasitic infection but it is more difficult to establish definitive values for clinical infection.

During the first week following anthelmintic treatment, serum gastrin concentrations appeared to follow a similar pattern to the improvement in appetite and liveweight performance, while serum pepsinogen values remained high for both infected groups. As the anthelmintic, albendazole, used has little effect on inhibited larvae, compared with others such as fenbendazole, pepsinogen concentrations took some time to fall (Mitchell, 1988) and indeed elevated values were still maintained 26 days post anthelmintic treatment, when the animals showed no visual symptoms of infection. Hence, in this respect, gastrin measurement at least in the first week seemed to parallel animal performance more closely than did pepsinogen.

In the later stages of the trial inorganic phosphorus concentrations declined in all infected animals. Similar results were recorded by Poppi (1988) who suggested that the established infection reduces phosphorus absorption from the gut with a consequent effect on circulating phosphorus concentrations. Levels were restored within a week of anthelmintic treatment indicating a rapid repair of the gut phosphorus absorption mechanism. These effects were not influenced by Co status with both infected groups showing a similar response.

As reported by MacPherson et al (1987) neutrophil function was unaffected by Ostertagia ostertagi infection and only showed differences due to Co treatment. Michel and Sinclair (1969) demonstrated

that the regulatory mechanisms governing the extent of Ostertagia ostertagi infection in calves was immunologically controlled. Therefore, the greater severity of infection seen in the Co-depleted cattle may have been due to the impaired immune response of the abomasal mucosa.

Mean serum vitamin B_{12} and MMA concentrations were not significantly influenced by Ostertagia ostertagi infection. Previous workers (MacPherson et al, 1987) also observed that serum vitamin B_{12} values were not affected by the degree of parasitic infection but no-one has specifically examined the influence of Ostertagia on serum MMA. Although, in general, MMA concentrations purely reflected the animals' Co status, low values (<1.00 μmole l^{-1}) were detected for one depleted animal. This animal showed the most severe symptoms of parasitic infection and was virtually eating no feed. At this point propionate metabolism would have ceased with a consequent reduction in the amount of MMA produced. On treatment with anthelmintic, appetite was restored with a consequent increased requirement for propionate metabolism. This resulted in an elevation of MMA concentrations to around 3.00 μmole l^{-1}. Hence, this brings into question the reliability of MMA as an indicator of Co deficiency when appetite is reduced by parasitic infection or indeed by Co deficiency itself.

As in experiment 2, serum vitamin B_{12} measured by radio-assay, in general, produced higher values than those obtained using the microbiological technique and further points to the possible need to define diagnostic criteria for each method of determination. Further, serum vitamin B_{12} monitored by radio-assay demonstrated for the Co-supplemented cattle a more marked week to week variation

than did the values obtained microbiologically. The reasons why this occurred are unclear.

As the anthelmintic contained 2.7% cobalt sulphate, increased serum vitamin B_{12} and decreased MMA concentrations would have been expected. The Co-supplemented cattle produced very high (>500 ng l^{-1}) serum vitamin B_{12} values throughout the first week following treatment but probably more as a result of the vitamin B_{12} injection given on the same day as the Co-supplemented anthelmintic. On the other hand, the Co-depleted animals produced only a very transient increase in serum vitamin B_{12} contents which had totally disappeared by one day following the initial treatment. MMA concentrations, however, showed no response to Co supplementation, suggesting that propionate metabolism remained impaired. Therefore, the addition of Co to the anthelmintic appeared to be an ineffective means of supplying supplementary Co to cattle. Although the rumen microbial population quickly adapted to the flush of Co to increase vitamin B_{12} synthesis initially, the amount supplied was insufficient to maintain this.

CHAPTER 8 - EVALUATION OF THE EFFICACY OF COBALT SUPPLEMENTATION OF AN ANTHELMINTIC

8.1 INTRODUCTION

In the past oral dosing with Co, although proving to be a successful method of treating or preventing Co deficiency has, until recently, been largely ignored as a routine method of Co-supplementation. As Co storage within the ruminant is limited, the treatment must be repeated at regular intervals to ensure deficiency symptoms do not appear (Underwood, 1981) with the frequency depending on both the severity of the condition and the dosage rate. For severely deficient animals, a minimum of weekly administered treatments is needed, i.e. 7 mg Co per sheep or 70 mg per cow each week (Underwood, 1981), but for animals with sub-clinical Co deficiency monthly doses of 250 mg Co or 1000 mg Co for sheep and cattle respectively may be adequate (COSAC/SARI, 1982). However, even with monthly dosing, oral Co supplementation is still labour-intensive requiring frequent handling. Therefore, it has been recently suggested that oral Co could be administered to the ruminant by the addition of Co to an anthelmintic (Hoechst U.K., 1983, 1984). By using this method, no special handling is required with Co being administered during routine farming practice.

Two trials which involved grazing lambs were set up to assess the efficacy of a Co-supplemented anthelmintic, i.e. Panacur SC (suspension containing 25 mg fenbendazole, 0.4 mg Se and 0.95 mg Co ml^{-1}) (Hoechst U.K.). The trials were also designed to test that the amounts of Co and Se given in the anthelmintic were non-toxic. In addition, three further Co supplementation methods, namely repetitive Co drenching, vitamin B_{12} injection and the single administration of a slow-release Co bolus were used for

comparative purposes. Although the original aim of the work was to assess both the Co and Se content of Panacur SC, only the results relating to the effects of Co will be given in detail here.

8.2 MATERIALS AND METHODS

Trials were carried out at Site 1: Grainston Farm, Dunblane and Site 2: Orchardton Farm, Kirkcudbright, both known to have a history of sub-clinical Co deficiency. At both sites half-bred ewe lambs were used with the experimental treatments detailed in Table 8.1. The trials were undertaken in the autumn (September to December, 1985) using spring-born lambs bred on each farm. Panacur and Panacur SC treatments were administered at the manufacturers' prescribed dosage rates (dependent on the animal's weight) at the start of the trials and at monthly intervals thereafter. Cosecure (Coopers Animal Health), a slow-release Co, Cu, Se bolus, was administered at the start of the trial on site 1. This bolus is composed of a specially impregnated glass which when embedded in the reticulum slowly dissolves giving a continuous supply of Co, Cu and Se (U.K. Wellcome Foundation Ltd., 1984). At site 2 intramuscular injections of 1000 μg vitamin B_{12} (Rycovit vitamin B_{12} containing cyanocobalamin 250 μg ml^{-1}) or an oral 250 mg Co $head^{-1}$ drench (from a 25 mg Co ml^{-1} $CoSO_4.7H_2O$ solution) were given to the appropriate lambs initially and repeated at monthly intervals thereafter. In addition to Co supplementation via Panacur SC and the slow-release bolus, the lambs at site 1 grazed Co-treated pasture between days 28 and 57. Blood sampling was carried out at the start of each trial and at monthly intervals thereafter. Additional samples were collected two weeks following the monthly Panacur treatments, once at site 1 (day 70) and twice at site 2 (days 42 and 65) (Table 8.2). Liveweights were recorded monthly.

Table 8.1 Experimental treatments at each site

	Site 1	Site 2
Farm Name	Grainston Farm	Orchardton Farm
District	Dunblane	Kirkcudbright
Nat. Grid. Ref.	NN 758033	NX 807552
Initial liveweight (kg)	35	42
No. of lambs per treatment	20	20
Dosage rate (ml)	7.5	10
Treatments:		
Group 1	Control	Panacur (Pan)
Group 2	Panacur + Cosecure (Pan + Cos)	Panacur SC (Pan SC)
Group 3	Panacur (Pan)	Panacur + 1000 μg vitamin B_{12} (Pan + Vit B_{12})
Group 4	Panacur SC (Pan SC)	Panacur + 250 mg Co drench (Pan + Co)

The Co status was evaluated using serum vitamin B_{12} concentrations as measured by radio-assay and Se status by the concentration of the Se containing enzyme GSH-Px in whole blood lysates. Monthly checks were also made of haemoglobin, plasma Cu and Mg to ensure these remained adequate. The analytical methods were as given in Chapter 3.

8.3 RESULTS

8.3.1 Liveweight

At both sites, treatment had no effect on liveweight. For site 1, liveweight remained fairly constant throughout, ranging between an average of 34-38 kg, while at site 2, the mean liveweights for each group showed a progressive increase from 42 to 47 kg over the period of the trial.

8.3.2 Serum vitamin B_{12}

(a) Site 1

Serum vitamin B_{12} concentrations throughout the experiment, irrespective of treatment, remained above the threshold value of 400 ng l^{-1} (Table 8.2). Twenty-eight days after the initial treatment, the "Cosecure" bolus resulted in highly significant ($P < 0.001$) increases in serum vitamin B_{12} concentrations over all the other groups. Although administration of Panacur SC also resulted in greater serum vitamin B_{12} concentrations than the control and Panacur treatment, the differences were not significant. However, by day 57 the effects of Co treatment, whether by a "Cosecure" bolus or Panacur SC, were masked by the fact that the sheep had inadvertently grazed Co-treated herbage between days 28 and 57. Two weeks (day 70) after the

Table 8.2 Mean vitamin B_{12} concentrations (ng l^{-1}) of lambs at each site

Site 1						Site 2					
Day	Control	Pan + Cos	Pan	Pan SC	SED	Day	Pan	Pan SC	Pan + Vit B_{12}	Pan + Co	SED
0	446	447	409	459	45	0	520	501	486	492	47
28	699	1036	649	717	67	28	392	449	490	457	32
57	788	867	817	860	69	*42	524	615	610	630	43
*70	-	-	651	848	68	55	612	768	748	752	56
84	510	627	487	576	52	*65	683	795	902	913	61
120	908	1123	967	990	59	77	652	648	935	873	61

*intermediate sampling

return to Co-deficient pasture, the administration of Panacur SC at day 57 resulted in significantly ($P < 0.001$) higher serum vitamin B_{12} concentrations than obtained for those given Panacur only. However, after a further 14 days (day 84) despite Panacur SC maintaining higher serum vitamin B_{12} concentrations than the Panacur and control groups, the differences were no longer significant. On the other hand, the "Cosecure" treatment resulted in significantly higher values than the control ($P < 0.05$) and Panacur only group ($P < 0.01$). This remained the case until the end of the trial 120 days after the start.

(b) Site 2

As at site 1, the initial serum vitamin B_{12} concentrations were within the limit of adequacy, i.e. ≥ 400 ng l^{-1} (Table 8.2). However, by day 28 the serum vitamin B_{12} concentrations of the Panacur group had fallen to <400 ng l^{-1}. Although concentrations also declined in the Panacur SC and Co groups (by 51 and 34 ng l^{-1} respectively compared to 128 ng l^{-1} for Panacur), the fall was significantly much smaller ($P < 0.001$: SED = 26.1). From days 0 to 42, the increase in serum vitamin B_{12} concentrations was significantly greater ($P << 0.001$: SED = 34.8) for all the groups given Co or vitamin B_{12} than for the Panacur only treatment. By day 55, the same three Co-treated groups gave mean serum vitamin B_{12} concentrations significantly above ($P < 0.05$) that of the Panacur group. While serum vitamin B_{12} levels were enhanced by around 250 ng l^{-1} 14 days after treatment at day 55, for the groups given the Co drench or vitamin B_{12} injection, Panacur SC only increased concentrations by

approximately 100 ng l^{-1} over the Panacur only group. Further, by day 77, both Panacur and Panacur SC groups produced similar vitamin B_{12} concentrations which were significantly ($P < 0.01$) lower than those obtained for either the Co drench or vitamin B_{12} injection treatments.

8.3.3 Glutathione Peroxidase (GSH-Px)

At both sites initial GSH-Px concentrations were below the deficiency concentration of 30 U ml^{-1} cells, but more markedly so at site 2 (Table 8.3). Within one month of the first treatment, Panacur SC increased GSH-Px significantly above that of the controls ($P < 0.01$ at site 1, $P < 0.001$ at site 2). Continued monthly treatment enhanced GSH-Px concentrations to satisfactory levels of around 100 U ml^{-1} cells at site 1 and 50 U ml^{-1} cells at site 2 by the end of the experiment. The administration of Se as a slow-release bolus at site 1 despite producing a similar improvement in Se status by the end of the trial took longer (57 days) to show a significant effect.

8.4 DISCUSSION

Panacur SC, although being of benefit as a Se source was generally not as successful a means of Co supplementation as either a slow-release Co bolus, Co drench or vitamin B_{12} injection. This was also found to be the case by Field et al (1988) who reported that a similar product, Ovitelmin S and C (mebendazole suspension containing 0.34 mg Se ml^{-1} and 0.44 mg Co ml^{-1}), was an effective means of supplying Se but that monthly administrations failed to sustain the improved serum vitamin B_{12} concentrations obtained after 4 to 7 days following treatment. In this present study, despite Panacur

Table 8.3 Mean GSH-Px concentrations (U ml^{-1} cells) at the different sites

Site 1						Site 2					
Day	Control	Pan + Cos	Pan	Pan SC	SED	Day	Pan	Pan SC	Pan + Vit B_{12}	Pan + Co	SED
0	26.3	29.2	26.4	28.6	4.6	0	11.2	13.3	18.2	18.8	2.3
28	33.5	39.8	27.4	46.1	4.8	28	30.3	44.4	30.4	29.5	2.1
57	47.6	84.6	47.1	84.2	6.0	55	32.9	55.3	26.7	28.0	4.5
84	40.2	82.1	39.6	85.0	5.7	77	18.8	52.3	25.3	21.5	3.3
120	42.7	100.0	42.4	106.0	9.2						

SC failing to improve serum vitamin B_{12} concentrations significantly over controls one month after treatment, samples taken at intermediate two-weekly sampling dates (day 70 at site 1 and days 42 and 65 at site 2) showed that the product could significantly improve Co status for at least 2 weeks after treatment. Hence, monthly administration will produce an initial boost in serum vitamin B_{12} concentrations, followed by a fall until the next treatment date when levels will rise again. Under sub-clinical Co conditions, these short periods of elevated serum vitamin B_{12} concentrations may be enough to prevent any loss in productivity but with clinical Co deficiency such a method of treatment will be inadequate. Further, from the evidence presented here there appears to be no risk of toxicity problems from the trace element inclusion rates. In fact, monthly dosing with 250 mg Co at site 2 significantly ($P < 0.001$) improved serum vitamin B_{12} concentrations over the controls with no toxicity problems. Such an amount is some 34 times greater than the amount found in Panacur SC and suggests that Co concentrations in Panacur SC could be safely increased to values which could maintain serum vitamin B_{12} levels between monthly treatments.

In terms of the other Co treatment methods, the administration of either a slow-release bolus, monthly 1000 µg vitamin B_{12} injections or monthly 250 mg Co drenches all proved to be successful means of significantly improving serum vitamin B_{12} concentrations over the controls. However, at site 1, access to pasture which had been treated with Co appeared to have a much greater effect in elevating serum vitamin B_{12} concentrations than either of the Co treatments used at this site (slow-release bolus or Panacur SC) and on this farm such a method of Co-supplementation may be the most cost-

effective supplementation method. On the other hand, at site 2, monthly treatments with 1000 μg vitamin B_{12} or 250 mg Co led to improved serum vitamin B_{12} levels. These findings coincide with those given by COSAC/SARI (1982) who suggest that large monthly treatments with either vitamin B_{12} or Co are effective provided the degree of Co deficiency is not too severe and no symptoms of clinical deficiency are seen.

Throughout the period of these trials serum vitamin B_{12} concentrations remained adequate ($\geq$400 ng l^{-1}) and particularly at site 2 where a progressive rise with time was seen, probably as a result of increased soil ingestion when pasture supply became limiting in late autumn. For a true appraisal of the various Co treatment methods further studies are required over a summer grazing period when Co demand is greater but supply is less.

CHAPTER 9 - AN INVESTIGATION INTO THE EFFICACY OF COBALT TREATMENT OF PASTURE IN TERMS OF INCREASING THE COBALT STATUS OF BOTH HERBAGE AND THE GRAZING RUMINANT

9.1 INTRODUCTION

In the past, various workers (Andrews, 1953; Reith and Mitchell, 1964; Poole et al, 1972; McLaren et al, 1979; Reith et al, 1983; Fleming and Parle, 1984; Evans, 1985; Klessa et al, 1988) have studied the influence of Co application to pasture as a means of elevating herbage Co contents. While Reith et al (1983) demonstrated that the application of 0.6 kg Co ha^{-1} as hydrated cobalt sulphate increased herbage Co concentrations for between 3 to 5 years, others (Evans, 1985; Klessa et al, 1988) found herbage Co values had fallen to pre-treatment contents after 1-2 years. Such variations in response have been attributed to soil pH (Nicolls and Honeysett, 1964), high soil manganese contents (Adams et al, 1969; Evans, 1985) while McLaren et al (1987) suggested, more generally, that plant uptake of applied Co is related to the ability of a soil to adsorb Co.

Whereas the influence of Co treatment of pasture has received substantial interest as a means of increasing herbage Co contents, less work has been carried out in relation to its effect on the grazing ruminant. Instead, most workers have assessed the residual value of Co treatment by taking regular cuts of grass over a specific period of time, following Co application to pots (Nicolls and Honeysett, 1964; Adams et al, 1969) or field plots (Reith and Mitchell, 1964; Adams et al, 1969; McLaren et al, 1979; Reith et al, 1983; Klessa et al, 1988). From such studies, predictions have been made as to the likely benefits of such treatments to the grazing

ruminant, without taking into account how animal factors, such as soil ingestion, might influence what actually happens in the animal. In contrast, other researchers (Stewart et al, 1955; O'Moore, 1957; Whitelaw and Russel, 1979) investigated Co pasture treatment in terms of improved Co status of grazing sheep but paid little attention to its effect on herbage Co concentrations.

Further, whereas various authors (Voss and MacPherson, 1977; COSAC/SARI, 1982) state that Co deficiency can be diagnosed from soil and herbage Co concentrations and from various blood components, there appears to be little information available which compares the relative merits of these parameters. Instead researchers have concentrated on either refining the methodology used or proposing better definitive criteria for use with soil, herbage or blood measurements.

Hence, the aims and objectives of this work were threefold, namely:

1. To examine how Co application to pasture influences soil Co availability, plant uptake and the Co status of the grazing ruminant.
2. To assess the merits of the various diagnostic criteria commonly used to predict Co deficiency.
3. To compare the cost-effectiveness of Co treatment of pasture with two direct methods of Co supplementation of the animal, namely regular vitamin B_{12} injections and the administration of a slow-release Co bolus at the start of the grazing season.

In order to carry out these aims and objectives, an animal trial was established on a site known to have a history of Co deficiency. As upland improvement schemes have been highlighted as potential

situations where Co deficiency can arise (COSAC/SARI, 1982), a recently upgraded field on the farm was chosen for the study. After splitting the field in two, one half received an application of Co at a rate recommended by COSAC/SARI (1982). Over two summers for both pastures, the Co statuses of the soil, herbage and the grazing ewes and twin lambs were monitored at regular intervals. In addition, for comparative purposes, one lamb from each pair on the untreated pasture received monthly vitamin B_{12} injections in 1986 and a slow-release Co bolus at the start of the grazing season in 1987.

9.2 MATERIALS AND METHODS

9.2.1 Site

The experiment was carried out on a 5 hectare field at Upper Auchinlay Farm, near Dunblane (Grid Reference NN767028), between April 1986 and September 1987. Three years previously, the farmer had upgraded the site by the application of lime and re-seeding to produce a perennial ryegrass and clover dominant sward.

Details of the parent materials and soil properties of the area are given in Table 9.1.

9.2.2 Experimental plan

In spring 1986 the field was split in two as shown in Figure 9.1, and on 18/4/86 when the grass height was short the appropriate half was treated with 0.6 kg Co ha^{-1} (as hydrated cobalt sulphate) applied as a spray using an "Allman" sprayer. As detailed in Chapter 6, the Co solution was filtered before application to remove the solid impurities.

Table 9.1 Parent material and properties of soils at the trial site at Upper Auchinlay Farm

(a) Parent material and soil type

Parent material	Soil association	Soil series	Drainage status
Lower old red sandstone sediments, sandstone flags and mud stones with some Highland Schist erratics	Balrownie	Dunblane	Free

(b) Soil properties

pH	% loss on ignition	% sand	% silt	% clay	Available Co mg kg^{-1}	Exchangeable Mn mg kg^{-1}	Reducible Mn mg kg^{-1}	Total Mn mg kg^{-1}	Available P mg l^{-1}	Available K mg l^{-1}	Extractable Mg mg l^{-1}
6.2	11.3	38.1	30.8	31.3	0.34 (L)	8.20	199	660	30 (M)	142 (M)	322 (H)

L = low; M = moderate; H = high status

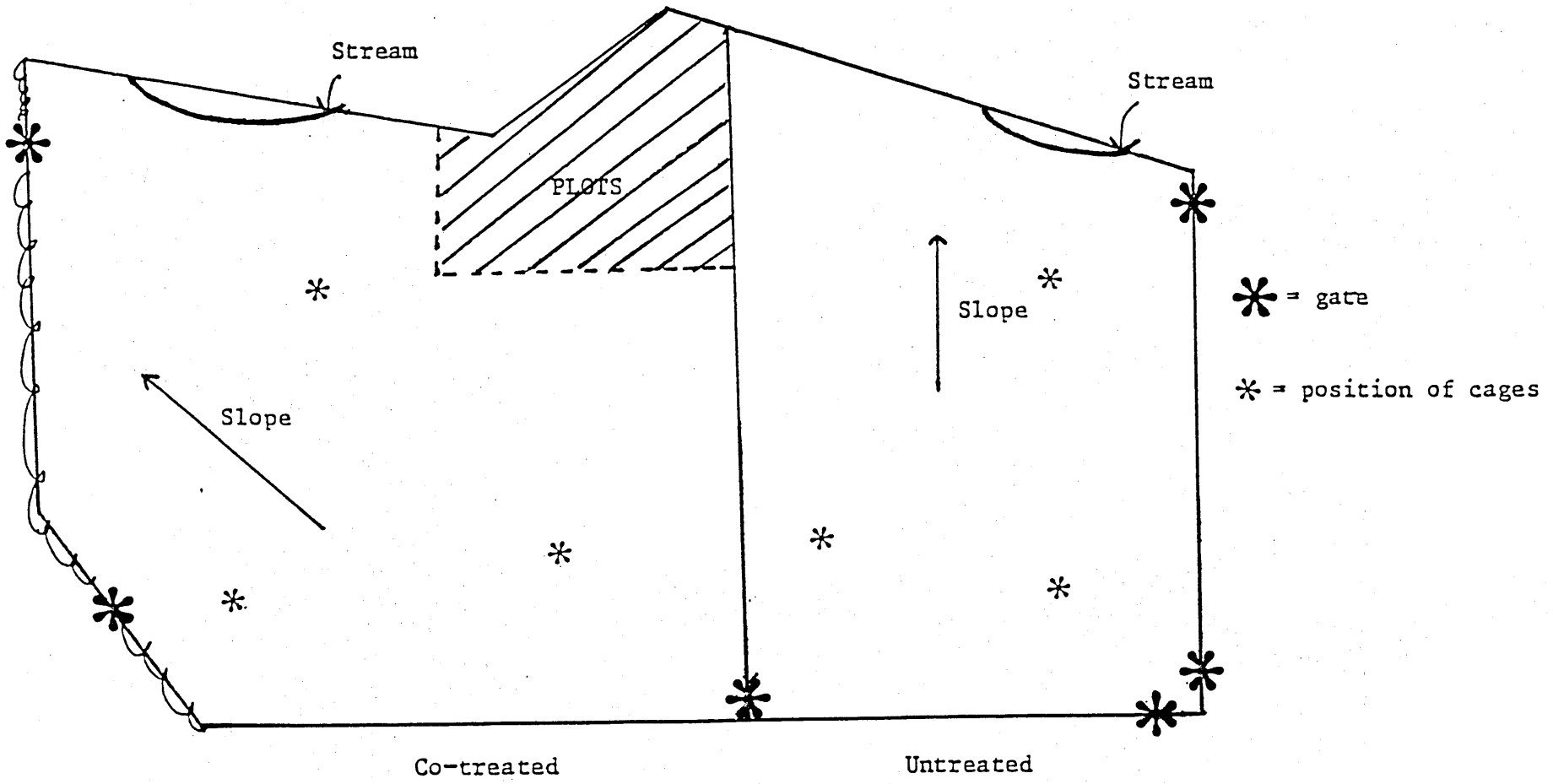
Before the sheep were introduced onto the pastures in 1986, three galvanised 1.8 m x 0.6 m x 1.0 m cages were placed at random on both areas as shown in Figure 9.1 and secured to the ground by metal hooks.

On 12/5/86, fifteen ewes with their twin spring-born lambs were introduced to each pasture with each group kept on their appropriate area until weaning on 5/8/86. As the farmer used a variety of sheep breeds, namely Blackface, Cheviot, Border Leicester, Charollais and Half-bred, the ewes were allocated to the treatments in such a way as to equalise the groups in terms of breed. One lamb from each pair on the untreated pasture was given a monthly 1000 µg vitamin B_{12} (hydroxocobalamin BP, Glaxovet).

The experiment recommenced the following year on 11/5/87 when another group of fifteen ewes with their twin spring-born lambs were introduced to each pasture as in the previous year, with the different breed types randomised between the treatment groups as before. As in 1986, one lamb from each pair on the untreated pasture was given a 1000 µg vitamin B_{12} injection on 11/5/87, followed by the administration of a slow-release glass bolus (Cosecure, Coopers Animal Health) on 15/6/87, once the rumen had become fully functional.

In both years a similar grazing height was maintained for each sward by rotating a group of young cattle between the two pastures.

Figure 9.1 Layout of Field Site



9.2.3 Parameters measured

(a) Soil

Soil samples were taken at the beginning and end of each grazing season by the methods detailed in Chapter 3. On each sample, pH, % loss on ignition and available Co was determined by the procedures given in Chapter 3.

(b) Herbage

Monthly herbage samples were collected during the summer months in 1986 and 1987. At each sampling date a total of three samples were taken from each pasture, namely:

(i) sheep shear cut;

(ii) electric shear cut;

(iii) a representative sample from the grass growing in the cages, using the electric shears used for (ii).

In 1986, using the sampling procedures given in Chapter 3, Co and Ti were determined using d.c. arc spectroscopy, while in 1987, Co and Fe were measured by inductively coupled plasma (I.C.P.) spectroscopy (Chapter 3).

(c) Animals

Both liveweights and serum vitamin B_{12} concentrations (radio-assay procedures) were monitored monthly during each grazing season using the techniques given in Chapter 3. Random checks on haemoglobin, plasma Cu and Mg and the Se enzyme, glutathione peroxidase, were determined on a representative number of samples taken on 9/6/86, 7/7/86, 5/8/86, 15/6/87 and 6/7/87 using the methods given in Chapter 3.

9.3 RESULTS

9.3.1 Soil Co concentrations

Co treatment of pasture had no measurable effect on available Co concentrations as assessed by 2.5% acetic acid (Table 9.2). The mean value of 0.43 mg Co kg^{-1}, using the MISR/SAC (1985) interpretative criteria, indicated that the soil was likely to produce Co-deficient herbage.

9.3.2 Herbage Co contents

During 1986, all herbage from the Co-treated pasture, irrespective of how the sample was taken, contained higher Co concentrations than those from the untreated pasture (Table 9.3). In addition, untreated pasture between June and August produced herbage containing <0.10 mg Co kg^{-1} DM, whilst Co treatment maintained herbage Co contents >0.10 mg kg^{-1} DM. This occurred despite the rapid fall in Co concentration of Co-treated pasture during the grazing season.

However, by 1987, the herbage Co concentrations of the Co-treated pasture had fallen to values which were very similar or only slightly above those of untreated pasture. In general, both pastures produced herbage containing <0.10 mg Co kg^{-1} DM, with very low Co concentrations in the range 0.02-0.06 mg Co kg^{-1} DM monitored for the untreated pasture in July.

On average, for both years, the grass samples taken with the electric shears contained more Co, Ti (in 1986) and Fe (in 1987) than those sampled using the hand shears, indicating a greater soil contamination of the electric shear cut samples. When the Co concentrations of the grass from the

Table 9.2 Results of soil analysis (trial site at Upper Auchinlay Farm)

Date	Available Co (mg Co kg^{-1})		pH		% loss on ignition	
	Untreated ground	Co-treated ground	Untreated ground	Co-treated ground	Untreated ground	Co-treated ground
10/3/88	0.34		6.0		11.3	
4/7/88	0.44	0.35	6.2	6.2	12.4	10.4
11/5/88	0.54	0.51	6.2	6.3	12.9	11.2
17/8/88	0.42	0.44	6.2	6.3	12.8	10.3

Table 9.3 Herbage Co and Ti (Fe) contents from cuts taken in 1986 and 1987 at Upper Auchinlay Farm

	Samples from untreated pasture			Samples from Co-treated pasture		
	Hand shears	Electric shears	Cages	Hand shears	Electric shears	Cages
12/5/86						
Co (mg kg^{-1})	0.12	1.28	ND	7.06	6.57	ND
Ti (mg kg^{-1})	40.0	799.0	ND	44.6	558.0	ND
9/6/86						
Co (mg kg^{-1})	0.10	0.09	0.06	0.77	1.88	0.81
Ti (mg kg^{-1})	19.6	20.9	7.5	20.3	47.0	9.5
7/7/86						
Co (mg kg^{-1})	0.05	0.08	0.06	0.27	0.29	0.35
Ti (mg kg^{-1})	14.1	16.1	12.9	21.6	16.2	13.9
5/8/86						
Co (mg kg^{-1}	0.05	0.08	0.07	0.14	0.16	0.28
Ti (mg kg^{-1})	11.9	25.0	11.7	10.3	12.6	16.8
11/5/87						
Co (mg kg^{-1})	ND	ND	0.06	ND	ND	0.16
Fe (mg kg^{-1})	ND	ND	196	ND	ND	233
15/6/87						
Co (mg kg^{-1})	0.07	0.13	0.03	0.07	0.12	0.06
Fe (mg kg^{-1})	180	192	77	115	185	88
6/7/87						
Co (mg kg^{-1})	0.05	0.06	0.05	0.08	0.09	0.05
Fe (mg kg^{-1})	114	135	111	120	139	98
30/7/87						
Co (mg kg^{-1})	0.02	0.05	ND	0.05	0.08	0.02
Fe (mg kg^{-1})	130	159	ND	92	123	80
17/8/87						
Co (mg kg^{-1})	0.05	0.07	0.08	0.06	0.07	0.04
Fe (mg kg^{-1})	114	180	219	122	152	121

Note: ND = not determined as insufficient sample available

cages was compared with the results from the rest of the field, as sampled with either hand or electric shears, no consistent trends were seen (Table 9.3).

9.3.3 Liveweight

In both years, grazing Co-treated pasture had no significant effect on the liveweights of ewes recorded at any sampling date (Table 9.4). However, it led to a small (2 kg) improvement in the overall liveweight gain in both grazing seasons, when compared with that of ewes given access only to untreated pasture (Table 9.5).

Similar results were obtained for the lambs, with Co treatment of pasture increasing the overall liveweight gains over the grazing season by 0.8 kg in 1986 and 1.7 kg in 1987 compared with the controls (Table 9.5). However, in 1986, monthly vitamin B_{12} injections improved liveweight gains by a further 1.4 kg above that of lambs grazing Co-treated pasture. In contrast, the administration of the slow-release bolus in 1987 produced an almost identical response in liveweight performance to that of lambs grazing pasture treated with Co.

9.3.4 Serum vitamin B_{12} concentration

In 1986, grazing Co-treated pasture significantly ($P < 0.001$) increased serum vitamin B_{12} concentrations of both ewes and lambs above the controls, and led to serum vitamin B_{12} levels of >2000 ng l^{-1} one month after introduction to the Co-treated pasture (Table 9.6). Although these values declined during the remainder of the grazing season, they always remained >1000 ng l^{-1}. Serum vitamin B_{12} concentrations of the controls also fell between June and September to finish just above the deficiency

Table 9.4 Mean monthly liveweight (kg) of sheep from trial site at Upper Auchinlay Farm (± S.E.)

Date	Dam		Lamb		
	Untreated pasture (n=15)	Co-treated pasture (n=15)	Untreated pasture (n=15)	Co-treated pasture (n=30)	Vitamin B_{12} or bolus (n=15)
12/5/86	46.3 ± 1.5	46.7 ± 1.5	6.4 ± 0.6	6.3 ± 0.3	7.1 ± 0.5
9/6/86	54.7 ± 1.8	56.2 ± 1.7	14.1 ± 0.8	14.6 ± 0.4	15.8 ± 0.9
7/7/86	58.9 ± 2.3	60.7 ± 1.9	21.7 ± 1.2	21.5 ± 0.5	23.4 ± 1.2
5/8/86	57.8 ± 1.9	60.5 ± 2.2	27.4 ± 1.4	28.1 ± 0.7	30.1 ± 1.4
11/5/87	54.1 ± 2.1	54.8 ± 2.0	10.6 ± 0.9	10.0 ± 0.5	10.8 ± 0.8
15/6/87	57.5 ± 1.7	58.7 ± 2.0	19.7 ± 1.1	19.6 ± 0.7	20.9 ± 1.2
6/7/87	60.7 ± 2.0	62.4 ± 2.0	24.9 ± 1.4	25.0 ± 0.9	26.2 ± 1.2
30/7/87	59.2 ± 2.0	63.1 ± 2.1	29.3 ± 1.5	29.9 ± 1.0	30.6 ± 1.6
17/8/87	60.9 ± 2.3	63.4 ± 2.2	30.8 ± 1.6	31.8 ± 0.9	31.7 ± 1.5

Table 9.5 Overall mean liveweight gains (kg) over each grazing season of sheep from experimental site at Upper Auchinlay Farm (± S.E.)

Year	Dam		Lamb		
	Untreated pasture (n=15)	Co-treated pasture (n=15)	Untreated pasture (n=15)	Co-treated pasture (n=30)	Vitamin B_{12} bolus (n=15)
1986	11.5 ± 0.9	13.8 ± 1.3	21.0 ± 1.0	21.8 ± 0.6	23.1 ± 1.1
1987	6.9 ± 1.3	8.6 ± 1.1	20.5 ± 1.0	22.2 ± 0.6	22.0 ± 1.2

Table 9.6 Mean monthly serum vitamin B_{12} (ng l^{-1}) concentrations of sheep from trial site at Upper Auchinlay Farm (± S.E.)

Date	Dam		Lamb		
	Untreated pasture (n=15)	Co-treated pasture (n=15)	Untreated pasture (n=15)	Co-treated pasture (n=30)	Vitamin B_{12} or bolus (n=15)
12/5/86	1080 ± 99	1170 ± 90	860 ± 94	870 ± 117	930 ± 150
9/6/86	890 ± 114	2270 ± 127	690 ± 123	2140 ± 108	930 ± 145
7/7/86	470 ± 55	1860 ± 88	240 ± 34	1630 ± 86	450 ± 74
5/8/86	450 ± 57	1670 ± 86	180 ± 14	1360 ± 61	520 ± 76
11/5/87	1080 ± 99	1010 ± 118	730 ± 130	740 ± 72	780 ± 127
15/6/87	790 ± 140	1140 ± 117	270 ± 37	590 ± 54	420 ± 62
6/7/87	600 ± 109	1000 ± 98	230 ± 25	640 ± 43	840 ± 103
30/7/87	590 ± 76	980 ± 93	290 ± 36	510 ± 36	780 ± 135
17/8/87	480 ± 64	990 ± 94	230 ± 37	480 ± 42	850 ± 95

threshold of 400 ng l^{-1} for the ewes, while the lambs produced values indicative of Co deficiency at both the July and August sampling dates (Table 9.6).

In 1987, after one month of grazing Co-treated pasture, serum vitamin B_{12} concentrations for both ewes and lambs were significantly ($P < 0.05$) increased above the controls (Table 9.6). Despite the values declining during the remainder of the grazing seasons, in the vitamin B_{12}-treated animals they always remained >400 ng l^{-1} for the lambs and >900 ng l^{-1} for the ewes. In contrast, the control lambs produced serum vitamin B_{12} concentrations of <400 ng l^{-1} from June onwards, while the control ewes maintained serum vitamin B_{12} levels just above adequacy, i.e. 400 ng l^{-1}.

Direct animal Co supplementation either by monthly vitamin B_{12} injections in 1986 or the administration of a slow-release bolus in 1987 produced serum vitamin B_{12} concentrations above that of the control and >400 ng l^{-1} (Table 9.6). In 1986, although regular vitamin B_{12} injections led to enhanced serum vitamin B_{12} concentrations, the values were always significantly ($P < 0.001$) lower than those obtained for lambs grazing the Co-treated pasture. On the other hand, the administration of the slow-release bolus in June 1987 significantly ($P < 0.05$) increased serum vitamin B_{12} levels above those of lambs grazing the Co-treated pasture from July onwards.

9.3.5 Random blood checks on haemoglobin, Cu, Mg and Se status

Throughout both years haemoglobin, plasma Cu and Mg and whole blood GSH-Px concentrations remained within the normal range for all the treatment groups.

9.4 DISCUSSION

Despite Co treatment of pasture having no effect on acetic acid extractable soil Co, herbage Co concentrations were substantially increased above the untreated pasture during the first year. This is at variance with other workers (Reith et al, 1983; Evans, 1985). where similar rates of Co application increased the extractable soil Co levels by 0.1-0.7 mg Co kg^{-1} soil. Further, Evans (1985) still obtained a difference of >0.1 mg available Co kg^{-1} soil between untreated and treated pasture five years after Co application despite finding no differences in the Co content of the respective pastures by this stage. However, McLaren et al (1979) only found a marked increase in extractable Co following cobalt sulphate application at two out of a total of four sites examined. McLaren et al suggest that this may be a result of the error involved in sampling an area which is large in relation to the increases in 'available' Co brought about by the Co treatment. In their work, McLaren et al calculated that a 0.6 kg Co ha^{-1} application will add 0.17 mg Co kg^{-1} to the topsoil. However, not all of this Co will enter the available pool of soil Co, for example, some Co will never reach the soil being retained on the foliage and consumed by the grazing animal, while a further proportion, which does enter the soil, will become bound in unavailable forms to various soil components. Hence, as stated by Mitchell (1964) the small additions of Co following cobalt sulphate treatment may be undetected by acetic acid extraction.

Co application to pasture only produced herbage Co concentrations in excess of 0.10 mg Co kg^{-1} during the first grazing season, with the values obtained in 1987 regarded as inadequate for the grazing ruminant. In the first year, very high herbage Co concentrations

were obtained one month after treatment as a result of foliar contamination. These declined during the following months as the herbage was consumed by the grazing sheep or as rain washed the adhering Co into the soil. However, it must be bourne in mind that even the samples taken with the hand shears, one month after the Co application (12/5/86), were subject to substantial soil contamination (a value of >10 mg Ti kg^{-1} taken as indicative of soil contamination problems) which would have falsely elevated the measured herbage Co contents.

Low residual values to Co application have also been obtained by others (McLaren et al, 1979; Evans, 1985; Klessa et al, 1988). Mitchell and Burridge (1979) found that liming reduced the uptake of Co from Co-treated plots and the relatively high pH of 6.2 at the Upper Auchinlay site may explain, at least partly, the poor response to Co application. However, Reith et al (1983) found a residual value of between 2-3 years following a similar application rate of Co to a mineral soil of pH 6.1-6.2. Hence, soil acidity is unlikely to be the sole explanation but may be a contributory factor increasing the reactivity of various soil components, such as the soil oxides to Co. McLaren et al (1986) suggested that soil oxides have a strong affinity for Co and can bind Co in unavailable forms. This was also found to be the case for Australian soils particularly in relation to manganese oxides where Adams et al (1969) suggested that soils of high total Mn (i.e. >1000 mg Mn kg^{-1}) were unlikely to respond to applied Co. Although the total Mn content at the Upper Auchinlay site was only 66% of this value, this in conjunction with a pH of 6.2 may account for the low residual value in terms of the poor Co uptake of the herbage in the second year.

In both years, grazing Co-treated pasture improved the liveweight performance of both ewes and lambs over the controls despite the herbage Co concentrations in 1987 being classed as deficient. In addition, Co application of pasture produced higher serum vitamin B_{12} levels than the controls and throughout both grazing seasons maintained values >400 ng l^{-1}. While serum vitamin B_{12} concentrations of the control lambs fell to around 200 ng l^{-1} in July and August 1986 and from July onwards in 1987, the control ewes always maintained serum vitamin B_{12} values within the adequate range, but at least 300 ng l^{-1} below those ewes grazing the treated pasture. The elevated serum vitamin B_{12} concentrations in 1986 found for sheep grazing Co-treated pasture can be accounted for in terms of the enhanced herbage Co contents of the treated pasture. However, this cannot be the reason for the 1987 results as both untreated and treated herbage contained almost similarly low Co concentrations. One possible way of explaining the results is in terms of soil ingestion. Despite acetic acid failing to show any difference in the soil Co content following Co treatment in samples taken to a depth of 15 cm, the top few centimetres below the soil surface may contain higher Co concentrations than the rest of the profile and substantially more than untreated pasture. In particular, this will be the case for a soil which has a large Co adsorbative capacity. For such soils, when the applied Co enters the soil it will be rapidly adsorbed and retained by various soil components near the soil surface. In the course of grazing, the ruminant will consume some of this Co enriched soil, along with herbage. According to Brebner and Suttle (1987) at least some of the Co in soil is available for microbial synthesis of vitamin B_{12} in the rumen and, hence, enhances the Co status of the animal. Soil ingestion is normally regarded as making a significant contribution to Co intake of

ruminants from late autumn through to the spring (Thornton, 1983), when grass is in short supply but it is normally regarded as being less important during the summer. However, McGrath et al (1982) found sheep could consume up to 400 g soil kg^{-1} body weight between May and early November. This will be at a maximum when the grass is kept short cropped as was seen in the early part of the summer of 1987 at Upper Auchinlay Farm. In fact grass growth was so poor in May 1987, when the sheep were introduced to the pastures, that no grass samples could be taken from the fields. During the remainder of the grazing season, grass supply was greater than was seen in May 1987 reducing soil intake and, hence, may account for the fall in serum vitamin B_{12} concentrations seen between June and August for both ewes and lambs on the Co-treated ground.

The findings of this trial call into question the validity of both soil and herbage Co analysis in the diagnosis of the likely Co status of grazing ruminants, particularly following Co application to pasture. The measurement of soil Co by the extractant acetic acid was unaffected in either year by Co treatment of pasture, and when the values obtained were interpreted using MISR/SAC (1985), the soils from both areas were classed as Co-deficient. Although herbage Co concentrations of samples taken in 1986 from the fields with either hand or electric shears correlated well with the serum vitamin B_{12} values of the ewes and lambs ($r = 0.98$, $P > 0.001$ for Co-treated pasture and $r = 0.874$, $P > 0.05$ for untreated pasture), non-significant relationships were seen in 1987. Further, in 1987 while both pastures, irrespective of cutting method, gave a herbage Co content <0.10 mg Co kg^{-1} (0.10 mg Co kg^{-1} being regarded by ARC (1982) as the minimum requirement for ruminants), ewes and lambs grazing the Co-treated pasture showed an improved Co status.

At all sampling dates, the samples taken with the electric shears led to greater soil contamination than those obtained by hand shears as assessed by either herbage Ti or Fe concentrations, with a consequent increase in the measured Co content of the herbage sample. However, these Co values did not show any improvement over those obtained with hand shears in relating the herbage Co content to the animals' Co status. Thus, despite the fact that soil ingestion may make a substantial contribution to the Co intake of grazing ruminants, soil contaminated herbage as obtained by electric shears does not give a measure of the Co available to the rumen micropopulation for the synthesis of vitamin B_{12}. This further suggests that electric shears do not mimic the grazing animal, and the soil removed by electric shears does not correspond to what is actually ingested by the grazing animal.

The Co concentration of herbages taken from the cages gave poorer correlation coefficients when regressed with the animals' serum vitamin B_{12} values than those obtained from the field by either the hand or electric shears. This exemplifies the statement of West (1981) that it is "a mistake to fence off part of a grazing stock field for sampling since the animal may graze selectively and, furthermore, cropping alters the plants' ability to take up trace elements from the soil."

In terms of increasing the liveweight production of the lambs, in 1986, monthly vitamin B_{12} injections proved to be the most effective method, but in 1987, the slow-release bolus and Co treatment of pasture both gave a similar response. However, in terms of cost, treatment by monthly vitamin B_{12} injections over five months for two years is almost four times as costly as either Co treatment of pasture or the administration of a slow-release bolus (Table 9.7).

Table 9.7 Comparative costs of Co treatment methods used at Upper Auchinlay Farm over a 2 year period

Treatment	Breakdown of cost (£)	Total cost for 2 years £	Remarks/benefits
Cobalt sulphate spray to pasture	Materials = £43 Labour = £25	68	No handling
Slow-release bolus	Each year: 15 x ewe bolus = £12 30 x lamb bolus = £15 Handling = £10	74	Single administration
Vitamin B_{12} injection (treating only during grazing season)	Each year: 15 x 2000 µg ewe injection for 5 months = £45 30 x 1000 µg lamb injection for 5 months = £45 Handling = £50	280	Repeated handling

Further, the injection regime necessitates frequent handling during a busy time in the farmer's year. Consequently the administration of a slow-release bolus or Co-application to pasture appear to be the most cost-effective methods of Co supplementation at Upper Auchinlay and similar farms.

CHAPTER 10

GENERAL DISCUSSION AND A LOOK TO THE FUTURE

At the experimental site at Upper Auchinlay Farm a combination of factors resulted in Co-deficient summer herbage (<0.10 mg Co kg^{-1}) for the untreated pasture in both 1986 and 1987, and the Co-treated pasture in 1987, namely:

(i) Inherent low soil Co concentrations

The parent material, Old Red Sandstone, of the area is inherently low in Co (Mitchell, 1960) and, on weathering, will produce soil containing little Co. The problem is further exacerbated as soil Co availability is reduced as a result of the free draining nature of the soil (Mitchell et al, 1957). Therefore, for these reasons COSAC/SARI (1982) defined the soil series, Dunblane, as being of moderate risk of producing low Co concentrations in summer herbage, and indeed during the two summers of the experiment, untreated pasture contained <0.10 mg Co kg^{-1}.

(ii) Various management schemes would reduce herbage Co concentrations

In addition to an inherent Co deficiency problem, various farming practices such as liming and fertiliser-N application will further reduce the availability of Co to the grazing ruminant. Although it was not shown conclusively in the grass species and pH pot trial (Chapter 5) that liming reduced soil Co availability, others (Mitchell, 1954; Wright and Lawton, 1954; McLaren et al, 1987; Klessa et al, 1988) have found that liming to a pH >6.0 has a detrimental effect on Co uptake. The experimental site at Upper Auchinlay Farm produced pH values in excess of pH 6.0 three to four years after lime application. However, it is difficult to assess how much this application altered the Co status of the

pasture since no data was available on either the original pH values or the herbage Co concentrations before lime was applied.

The farmer's policy at Upper Auchinlay Farm was to apply an annual dressing of 87 kg N ha^{-1} as ammonium nitrate in May. Field plots set up at the experimental site, as described in Chapter 6, showed that such a fertiliser-N treatment did reduce the herbage Co content of the pasture. However, in both 1986 and 1987 the experimental plots receiving no fertiliser-N and no Co treatment maintained herbage below 0.10 mg Co kg^{-1}, the recommended minimum value. Thus, even without fertiliser-N application, the herbage Co content would be inadequate for grazing sheep and some form of Co supplementation would be required to obtain maximum productivity. On Co-treated pasture, fertiliser-N application also reduced the Co concentration of herbage (Chapter 6). However, during the first summer following Co treatment (i.e. 1986) even after fertiliser-N treatment, herbage Co concentrations were maintained above the recommended guidelines. By the second summer (i.e. 1987), irrespective of whether fertiliser-N was applied, the herbage Co concentration was below 0.10 mg Co kg^{-1}. Hence, at Upper Auchinlay Farm, fertiliser-N application had little influence on determining whether the pasture contained sufficient Co for the grazing sheep. However, on other farms where untreated pasture contains borderline Co concentrations, fertiliser-N application could change a Co-adequate pasture to a Co-deficient pasture. The results from Chapter 6 suggest that this effect will be more pronounced if nitrochalk is used.

At the experimental site, the sward, which was established in 1983, consisted mainly of perennial ryegrass and clover. Since no data is available on the Co concentrations of the native sward it is

impossible to establish the extent to which the introduction of these grass species altered the Co status of the site. However, other workers (Voss and MacPherson, 1977) suggested such a change would have a detrimental effect on the Co content of the pasture. At any particular time, the herbage Co concentrations will not only be determined by the grass species, but factors such as stage of maturity and grazing regime, will also govern the Co content of the pasture.

From the results in Chapter 5, it appears that although the perennial ryegrass/clover rich sward at Upper Auchinlay Farm contained a greater Co content than would have been the case in a timothy rich sward, Co supplementation of the grazing ewes would still be required. Therefore, at this site, the choice of grass species in a re-seed mixture would be relatively unimportant since Co treatment of the sheep would still be required even when the most Co rich grasses were sown.

(iii) The strong adsorption of soil applied Co

From the findings in Chapter 4, the relatively high pH of 6.2 at the experimental site would appear to be the main reason for the poor residual value of the application of 0.6 kg Co ha^{-1} as hydrated cobalt sulphate to pasture. Such a pH in conjunction with the clay loam texture and relatively high sesquioxide content (Total Mn = 660 mg Mn kg^{-1}, Total Fe = 3.1 x 10^4 mg Fe kg^{-1}) would result in a strong irreversible binding of the soil applied Co.

Hence, due to the inherently low Co content of soil at Upper Auchinlay Farm, herbage Co concentrations of untreated pasture were insufficient for grazing ruminants and produced serum vitamin B_{12} levels <400 ng l^{-1}

in lambs (Chapter 9). Various farm management factors (e.g. liming, fertiliser-N application and re-seeding) would further reduce Co availability to the grazing ruminant. However, it is impossible to establish the extent to which these factors altered the Co status of the pasture, i.e. whether their use changed the pasture from one of borderline Co status to a Co-deficient pasture.

At Upper Auchinlay Farm, Co supplementation resulted in a slight improvement in animal productivity as seen by the small increase in liveweight gains of ewes and lambs receiving Co. However, probably of more importance is the improved immune response following Co treatment. In Chapter 7, an improved immune status was obtained after Co administration to Co-depleted cattle, and Fisher and MacPherson (1986) found a similar effect in sheep. Hence, when Co intake is insufficient the reduced immune response exacerbates the direct effect of Co deficiency on animal productivity by increasing the incidence of infection and disease. This can result in a greater severity of gastro-intestinal parasitic infection as was found in Co-depleted cattle (Chapter 7) and the lowered viability of lambs born to Co-deficient ewes (Fisher and MacPherson, 1986).

At the experimental site, monthly vitamin B_{12} injections, administration of a slow-release bolus or Co treatment of pasture proved to be successful methods of increasing serum vitamin B_{12} concentrations above the deficiency threshold (Chapter 9). However, extrapolating these findings to other farms is difficult. Factors such as cost and feasibility will determine which method is the most suitable on any individual farm. At Upper Auchinlay Farm the farmer's policy is to use a Co-supplemented anthelmintic. From the results presented in Chapter 8, such a method will be of some benefit, but is unlikely to be as successful as monthly vitamin B_{12} injections, administration of a slow-release bolus or Co

treatment of pasture, in alleviating Co deficiency. By using one of these other treatment methods the farmer may see an improvement in animal productivity both as a direct Co effect and also from a reduced incidence of disease and infection.

From the work at Upper Auchinlay Farm and the other experiments various areas appear to merit further investigation, such as:

(i) Adsorption/desorption

1) A more detailed study looking at a greater range of soil types in order to determine more accurately the soil properties governing Co adsorption and desorption is merited. This is particularly required for Co desorption.

2) The production of one or more equations which can relate the properties of a soil to its likely response to applied Co.

(ii) Fertiliser-N application

Confirmation of the present findings is required together with investigations of the validity of some of the hypotheses advanced. For example, how do NO_3-N and NH_4-N influence Co uptake and what are the long-term effects of different forms and rates of fertiliser-N application on Co availability and the influence of these on changes in soil pH and clover content of a sward?

(iii) Diagnosis of Co status

1) An attempt should be made to obtain a better soil extractant which can predict more accurately the availability of both native and soil applied Co. When acetic acid was first proposed as an extractant analytical techniques were insensitive. However, with the advent of more sensitive instruments it should be possible to examine extractants which remove

smaller quantities of Co and more clearly mimic Co plant availability.

2) Further study into the use of serum MMA as a diagnostic marker of Co status in ruminants should be undertaken and diagnostic criteria defined. In particular, the effect of inappetence on serum MMA should be examined.

3) A comparison of serum vitamin B_{12} values obtained by the microbiological and radio-assay methods for cattle should be made and reasons for the differences that exist determined. If the findings of Chapter 7 are confirmed then separate interpretive criteria for both determinations should be drawn up.

(iv) Immune response relationship to Co status

The mechanisms behind the poor immune response of ruminants when dietary Co intake is low require elucidation. Further studies are required to relate the impairment in immune status to the enhanced susceptibility of Co-deficient ruminants to disease and infection.

(v) Prevention and treatment of Co deficiency

The effectiveness of Co pasture treatment as a means of preventing Co deficiency should be further investigated by examining how much of the enhanced Co status of the grazing ruminant can be attributed to ingestion of Co-enriched soil. This would require, among other things, an assessment of the distribution of the applied Co down the soil profile in different soil types.

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APPENDIX I Mean dry weight yields, and Zn, Mo, Mn and Fe concentration of herbage from grass species and pH pot trial (Chapter 5)

(a) Mean (n=4) dry weight yields (g pot^{-1}) of pH and species treatments from the grass species and pH pot trial (Chapter 5) (± S.E.)

(i) First cut

Nominal pH	Species						
	Mixture	White clover	Italian ryegrass	Perennial ryegrass	Timothy	Meadow grass	Red fescue
5.0	17.8 ± 1.0	22.7 ± 1.7	13.4 ± 1.3	14.6 ± 0.7	17.5 ± 0.9	6.8 ± 0.5	5.6 ± 1.0
5.5	14.9 ± 1.4	22.6 ± 1.4	13.2 ± 1.3	15.2 ± 2.8	13.0 ± 1.0	6.2 ± 1.3	5.4 ± 0.6
6.0	18.7 ± 1.2	19.1 ± 1.5	15.2 ± 0.8	13.8 ± 0.9	18.5 ± 1.6	3.6 ± 0.4	5.2 ± 0.5
6.5	19.2 ± 0.5	16.2 ± 0.8	18.8 ± 1.3	17.4 ± 1.3	20.0 ± 0.9	3.7 ± 0.3	8.5 ± 0.3

(ii) Second cut

Nominal pH	Species						
	Mixture	White clover	Italian ryegrass	Perennial ryegrass	Timothy	Meadow grass	Red fescue
5.0	17.0 ± 0.2	11.1 ± 1.0	15.4 ± 0.6	14.7 ± 0.6	14.0 ± 0.6	13.1 ± 0.8	12.2 ± 0.4
5.5	15.0 ± 0.4	12.7 ± 1.7	16.9 ± 0.4	15.5 ± 1.1	14.5 ± 1.0	11.6 ± 0.8	12.1 ± 1.2
6.0	16.6 ± 0.7	9.4 ± 0.5	19.3 ± 0.3	17.1 ± 1.4	17.0 ± 1.4	11.0 ± 0.6	10.1 ± 0.8
6.5	15.5 ± 0.8	6.8 ± 0.5	21.0 ± 0.9	15.4 ± 0.7	13.5 ± 1.0	11.5 ± 0.7	12.8 ± 1.0

(b) Mean (n=4) herbage Zn concentrations (mg Zn kg DM^{-1}) of pH and species treatments from the grass species and pH pot trial (Chapter 5) (± S.E.)

(i) First cut

Nominal pH	Species						
	Mixture	White clover	Italian ryegrass	Perennial ryegrass	Timothy	Meadow grass	Red fescue
5.0	33.2 ± 2.3	18.6 ± 0.7	26.8 ± 1.0	28.2 ± 3.0	32.5 ± 3.0	21.6 ± 1.1	17.4 ± 1.3
5.5	27.7 ± 0.4	18.1 ± 0.5	24.3 ± 0.5	26.1 ± 1.9	26.4 ± 0.9	23.4 ± 4.0	14.0 ± 0.5
6.0	21.5 ± 0.9	20.4 ± 0.9	23.0 ± 3.0	24.3 ± 0.7	23.4 ± 0.9	20.3 ± 3.0	17.3 ± 1.8
6.5	21.7 ± 0.9	23.1 ± 1.6	17.3 ± 1.1	19.9 ± 1.9	23.5 ± 1.4	18.9 ± 1.3	13.3 ± 1.5

(ii) Second cut

Nominal pH	Species						
	Mixture	White clover	Italian ryegrass	Perennial ryegrass	Timothy	Meadow grass	Red fescue
5.0	32.7 ± 1.9	36.4 ± 1.4	28.6 ± 0.9	30.8 ± 1.3	33.2 ± 1.5	24.4 ± 1.1	18.9 ± 0.3
5.5	28.4 ± 0.5	32.5 ± 2.0	23.5 ± 0.7	26.5 ± 1.7	26.4 ± 1.7	18.5 ± 1.9	15.5 ± 1.2
6.0	23.8 ± 1.7	28.0 ± 1.6	20.0 ± 0.9	21.4 ± 0.7	24.1 ± 0.8	18.7 ± 1.0	17.6 ± 1.1
6.5	21.5 ± 0.7	31.0 ± 1.6	16.4 ± 0.8	20.2 ± 1.4	23.2 ± 0.6	19.0 ± 0.8	14.5 ± 1.3

(c) Mean (n=4) herbage Mo concentrations (mg Mo kg DM^{-1}) of pH and species treatments from grass species and pH pot trial (Chapter 5) (± S.E.)

(i) First cut

Nominal pH	Species						
	Mixture	White clover	Italian ryegrass	Perennial ryegrass	Timothy	Meadow grass	Red fescue
5.0	0.71 ± 0.05	0.21 ± 0.02	0.67 ± 0.07	0.75 ± 0.02	1.81 ± 0.13	0.85 ± 0.05	0.35 ± 0.03
5.5	1.35 ± 0.05	0.25 ± 0.03	1.15 ± 0.15	1.16 ± 0.13	2.72 ± 0.20	1.54 ± 0.33	0.99 ± 0.10
6.0	1.78 ± 0.08	0.85 ± 0.13	1.96 ± 0.22	1.72 ± 0.17	2.64 ± 0.06	1.99 ± 0.17	1.33 ± 0.22
6.5	2.02 ± 0.04	1.13 ± 0.06	1.59 ± 0.14	2.00 ± 0.18	2.51 ± 0.15	1.33 ± 0.15	1.64 ± 0.17

(ii) Second cut

Nominal pH	Species						
	Mixture	White clover	Italian ryegrass	Perennial ryegrass	Timothy	Meadow grass	Red fescue
5.0	1.10 ± 0.07	0.61 ± 0.05	1.11 ± 0.11	1.18 ± 0.11	2.43 ± 0.25	2.01 ± 0.10	0.89 ± 0.07
5.5	1.80 ± 0.10	1.08 ± 0.16	1.43 ± 0.14	1.89 ± 0.14	3.28 ± 0.27	2.32 ± 0.19	1.60 ± 0.28
6.0	2.17 ± 0.23	2.34 ± 0.15	1.66 ± 0.04	1.96 ± 0.25	2.84 ± 0.14	3.56 ± 0.35	2.18 ± 0.15
6.5	2.92 ± 0.17	2.62 ± 0.40	1.59 ± 0.13	2.54 ± 0.15	3.23 ± 0.35	2.40 ± 0.38	2.18 ± 0.41

(d) Mean (n=4) herbage Mn concentrations (mg Mn kg DM^{-1}) of pH and species treatments from grass and pH pot trial (Chapter 5) (± S.E.)

(i) First cut

Nominal pH	Species						
	Mixture	White clover	Italian ryegrass	Perennial ryegrass	Timothy	Meadow grass	Red fescue
5.0	393 ± 15	480 ± 14	338 ± 9	236 ± 18	403 ± 11	149 ± 6	125 ± 11
5.5	364 ± 16	349 ± 24	261 ± 17	215 ± 19	499 ± 18	155 ± 20	219 ± 14
6.0	286 ± 20	337 ± 6	215 ± 7	164 ± 22	418 ± 53	145 ± 12	151 ± 11
6.5	262 ± 17	344 ± 4	262 ± 30	193 ± 16	462 ± 10	132 ± 17	171 ± 15

(ii) Second cut

Nominal pH	Species						
	Mixture	White clover	Italian ryegrass	Perennial ryegrass	Timothy	Meadow grass	Red fescue
5.0	359 ± 25	1055 ± 66	387 ± 11	299 ± 8	430 ± 47	229 ± 24	225 ± 20
5.5	513 ± 23	942 ± 100	441 ± 41	387 ± 25	642 ± 28	301 ± 85	352 ± 66
6.0	507 ± 25	767 ± 62	394 ± 26	298 ± 35	582 ± 102	262 ± 32	328 ± 12
6.5	522 ± 16	650 ± 42	319 ± 18	365 ± 17	536 ± 80	199 ± 7	389 ± 55

(e) Mean (n=4) herbage Fe concentrations (mg Fe kg DM^{-1}) of pH and species treatments from grass and pH pot trial (Chapter 5) (± S.E.)

(i) First cut

Nominal pH	Species						
	Mixture	White clover	Italian ryegrass	Perennial ryegrass	Timothy	Meadow grass	Red fescue
5.0	197 ± 32	246 ± 30	152 ± 8	117 ± 19	275 ± 27	210 ± 22	84 ± 12
5.5	231 ± 12	201 ± 22	112 ± 11	158 ± 40	161 ± 14	91 ± 17	90 ± 23
6.0	165 ± 20	245 ± 8	113 ± 11	95 ± 8	124 ± 22	89 ± 9	98 ± 22
6.5	139 ± 4	261 ± 13	102 ± 19	102 ± 8	99 ± 16	62 ± 5	54 ± 1

(ii) Second cut

Nominal pH	Species						
	Mixture	White clover	Italian ryegrass	Perennial ryegrass	Timothy	Meadow grass	Red fescue
5.0	92 ± 14	190 ± 29	97 ± 7	98 ± 22	100 ± 8	89 ± 17	59 ± 7
5.5	103 ± 5	168 ± 30	74 ± 12	115 ± 33	102 ± 19	74 ± 12	57 ± 4
6.0	83 ± 10	193 ± 25	60 ± 5	63 ± 7	80 ± 8	57 ± 5	82 ± 26
6.5	82 ± 2	154 ± 37	63 ± 8	60 ± 4	86 ± 7	50 ± 3	53 ± 3

APPENDIX II Herbage Fe concentrations (mg Fe kg DM^{-1}) for the different species grown at the two different sites investigated in the grass species field trial (Chapter 5)

Species	Balig		Sclenteuch	
	1st cut	2nd cut	1st cut	2nd cut
Clover	96	90	78	70
Italian ryegrass	107	121	44	53
Perennial ryegrass	115	90	72	88
Cocksfoot	92	72	47	73
Crested dog's tail	105	72	34	93
Timothy	94	73	61	87
Yorkshire fog	101	76	40	107
Meadow foxtail	91	95	ND	ND
Meadow grass	99	ND	36	117
Sweet vernal	70	ND	ND	ND
Poa compressor	ND	ND	56	129
Bent	ND	69	56	71
Red fescue	70	66	43	67
Vetch	ND	78	ND	ND

ND = not determined

APPENDIX III Mean herbage Fe, Mn, Cu, Zn and Mo concentrations for the fertiliser N plot trial at Upper Auchinlay Farm in 1986 and 1987 (Chapter 6)

(a) Mean (n=3) herbage Fe concentrations (mg Fe kg DM^{-1}) for fertilizer N plot trial (Chapter 6)

(i) 1986

Fertilizer N form	Total N applied kg N ha^{-1}	1st cut		2nd cut	
		Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}
Nitrochalk	0	78	86	97	84
	87	69	74	84	86
	174	76	83	87	82
	87*	76	83	94	100
Urea	0	86	85	95	113
	87	74	87	84	84
	174	77	87	85	87
	87*	77	87	94	110
Ammonium nitrate	0	81	88	104	92
	87	77	78	109	89
	174	76	71	95	111
	87*	76	71	82	94
S.E.		6.04		8.56	

*applied at start of each grazing season only

(ii) 1987

Fertilizer N form	Total N applied kg N ha^{-1}	1st cut		2nd cut		3rd cut	
		Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}
Nitrochalk	0	65	67	73	68	100	88
	130.5	55	56	83	83	77	85
	261	67	65	87	82	82	83
	87*	67	65	75	84	94	83
Urea	130.5	57	69	82	89	81	96
	261	63	66	94	91	84	82
	522	77	88	96	95	95	102
	87*	63	66	69	68	81	85
Ammonium nitrate	130.5	63	56	79	79	76	88
	261	70	63	87	82	98	103
	522	76	81	92	87	95	107
	87*	70	63	74	72	82	76
S.E.		5.30		5.08		6.70	

*applied at start of each grazing season only

(b) Mean (n=3) herbage Mn concentrations (mg Mn kg DM^{-1}) for fertilizer N plot trial (Chapter 6)

(i) 1986

Fertilizer N form	Total N applied kg N ha^{-1}	1st cut		2nd cut	
		Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}
Nitrochalk	0	62	68	101	89
	87	57	69	71	97
	174	68	74	90	103
	87*	68	74	94	94
Urea	0	74	60	71	87
	87	54	74	66	95
	174	66	61	84	70
	87*	66	61	98	90
Ammonium nitrate	0	70	83	98	102
	87	47	60	72	94
	174	75	64	114	102
	87*	75	64	103	74
S.E.		8.65		11.58	

*applied at start of each grazing season only

(ii) 1987

Fertilizer N form	Total N applied kg N ha^{-1}	1st cut		2nd cut		3rd cut	
		Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}
Nitrochalk	0	55	56	84	84	91	89
	130.5	53	49	85	94	90	107
	261	76	89	132	168	122	161
	87*	76	89	84	87	137	117
Urea	130.5	54	65	78	102	90	101
	261	65	62	127	128	121	135
	522	69	64	119	127	131	106
	87*	65	62	90	86	107	93
Ammonium nitrate	130.5	55	50	81	77	105	104
	261	84	71	149	140	172	139
	522	74	84	155	147	158	151
	87*	84	71	112	74	134	109
S.E.		7.50		19.38		18.58	

*applied at start of each grazing season only

(c) Mean (n=3) herbage Cu concentrations (mg Cu kg DM^{-1}) for fertilizer N plot trial (Chapter 6)

(i) 1986

Fertilizer N form	Total N applied kg N ha^{-1}	1st cut		2nd cut	
		Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}
Nitrochalk	0	5.72	6.35	6.81	6.94
	87	5.93	6.04	6.99	7.01
	174	6.78	6.63	8.91	8.44
	87*	6.78	6.63	6.42	6.62
Urea	0	6.29	5.76	6.74	6.67
	87	5.72	6.25	6.62	7.00
	174	6.35	6.38	7.85	7.86
	87*	6.35	6.38	6.75	6.69
Ammonium nitrate	0	5.78	5.96	6.79	6.69
	87	6.03	6.23	6.75	6.97
	174	6.83	6.45	7.98	8.24
	87*	6.83	6.45	6.47	5.69
S.E.		0.233		0.375	

*applied at start of each grazing season only

(ii) 1987

Fertilizer N form	Total N applied kg N ha^{-1}	1st cut		2nd cut		3rd cut	
		Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}
Nitrochalk	0	5.11	5.33	5.57	5.57	6.41	7.08
	130.5	7.12	6.55	8.70	8.47	9.27	9.81
	261	7.99	8.42	10.47	9.59	11.90	12.03
	87*	7.99	8.42	7.02	7.32	7.31	7.20
Urea	130.5	5.78	6.51	7.95	8.17	9.85	9.24
	261	7.13	7.40	9.75	9.98	11.23	11.48
	522	7.69	8.14	8.64	8.81	12.07	11.08
	87*	7.13	7.40	5.97	5.82	6.84	7.09
Ammonium nitrate	130.5	6.29	6.66	8.22	8.31	10.55	10.15
	261	7.92	7.20	9.70	9.52	11.75	11.99
	522	7.68	8.23	8.98	9.54	12.17	11.82
	87*	7.92	7.20	7.75	6.46	6.90	6.61
S.E.		0.346		0.512		0.393	

*applied at start of each grazing season only

(d) Mean (n=3) herbage Zn concentrations (mg Zn kg DM^{-1}) for fertilizer N plot trial (Chapter 6)

(i) 1986

Fertilizer N form	Total N applied kg N ha^{-1}	1st cut		2nd cut	
		Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}
Nitrochalk	0	20.4	21.6	25.5	24.6
	87	22.6	22.6	29.5	29.3
	174	25.1	25.0	34.1	33.7
	87*	25.1	25.0	25.1	28.5
Urea	0	22.4	20.0	24.5	23.7
	87	20.5	25.0	26.3	27.8
	174	23.5	23.4	31.1	31.1
	87*	23.5	23.4	25.3	26.5
Ammonium nitrate	0	21.7	20.0	24.2	23.3
	87	22.9	24.0	27.5	27.6
	174	25.3	23.8	31.8	33.8
	87*	25.3	23.8	26.3	24.1
S.E.		0.93		1.17	

*applied at start of each grazing season only

(ii) 1987

Fertilizer N form	Total N applied kg N ha^{-1}	1st cut		2nd cut		3rd cut	
		Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}
Nitrochalk	0	23.1	23.7	24.7	26.5	27.9	28.8
	130.5	27.2	26.1	31.8	34.1	32.8	35.8
	261	29.9	30.4	34.3	33.1	28.8	31.8
	87*	29.9	30.4	27.6	29.2	30.9	31.7
Urea	130.5	25.9	26.5	31.3	33.5	33.0	35.1
	261	28.8	30.0	34.8	32.3	31.9	31.3
	522	31.5	29.8	33.9	32.4	36.3	33.1
	87*	28.8	30.0	25.9	26.3	28.1	29.2
Ammonium nitrate	130.5	28.9	26.7	31.2	30.7	33.0	34.8
	261	30.2	29.1	32.4	32.2	33.3	33.1
	522	32.1	33.2	36.7	36.6	37.8	38.9
	87*	30.2	29.1	30.1	26.2	28.9	28.6
S.E.		1.24		1.77		1.69	

*applied at start of each grazing season only

(e) Mean (n=3) herbage Mo concentrations (mg Mo kg DM^{-1}) for fertilizer N plot trial (Chapter 6)

(i) 1986

Fertilizer N form	Total N applied kg N ha^{-1}	1st cut		2nd cut	
		Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}
Nitrochalk	0	0.81	0.73	1.04	0.84
	87	0.69	0.74	0.82	0.88
	174	0.76	0.66	0.95	0.77
	87*	0.76	0.66	0.96	0.99
Urea	0	0.71	0.65	0.89	1.01
	87	0.69	0.75	1.04	0.98
	174	0.75	0.81	0.95	1.14
	87*	0.75	0.81	0.90	0.88
Ammonium nitrate	0	0.75	0.58	0.91	0.89
	87	0.82	0.77	0.99	1.02
	174	0.78	0.78	0.95	0.95
	87*	0.78	0.78	0.78	0.98
S.E.		0.077		0.117	

*applied at start of each grazing season only

(ii) 1987

Fertilizer N form	Total N applied kg N ha^{-1}	1st cut		2nd cut		3rd cut	
		Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}	Untreated	Treated with 0.6 kg Co ha^{-1}
Nitrochalk	0	0.76	0.57	1.16	0.65	1.48	1.12
	130.5	0.74	0.71	1.12	1.07	1.26	1.24
	261	0.65	0.60	0.96	0.72	0.99	0.65
	87*	0.65	0.60	0.66	0.82	0.99	1.22
Urea	130.5	0.61	0.69	0.94	0.91	1.27	1.11
	261	0.75	0.71	0.92	1.02	1.01	0.99
	522	0.71	0.70	0.62	0.71	0.73	0.94
	87*	0.75	0.71	1.08	0.71	1.32	1.16
Ammonium nitrate	130.5	0.83	0.94	1.09	1.25	1.11	1.21
	261	0.61	0.66	0.90	0.73	1.17	0.93
	522	0.55	0.53	0.62	0.57	0.89	0.77
	87*	0.61	0.66	0.85	0.97	1.00	1.15
S.E.		0.109		0.167		0.215	

*applied at start of each grazing season only

APPENDIX IV Mean concentrations of the various parameters determined for the cattle in Experiments 1, 2 and 3 described in Chapter 7

(a) Mean concentrations of the various blood parameters determined for the six cattle in Experiment 1 (Chapter 7) over the whole trial (± S.E.)

(i) Co depletion (6 cattle)

Blood parameter	No. of samples	Trial mean
Haemoglobin (g 100 ml^{-1})	152	10.29 ± 0.10
Plasma glucose (mg %)	151	78.4 ± 0.56
Alkaline phosphatase (U l^{-1})	151	214.1 ± 3.96
Aminotransferase (ST ml^{-1})	91	54.4 ± 0.74
GSH-Px (U ml^{-1} cells at 30°C)	152	27.6 ± 1.17
Plasma Ca (m moles l^{-1})	151	2.54 ± 0.01
Plasma Mg (m moles l^{-1})	151	0.99 ± 0.004
Plasma Cu (μ moles l^{-1})	151	11.83 ± 0.13

(ii) Co repletion (4 cattle)

Blood parameter	No. of samples	Trial mean
Haemoglobin (g 100 ml^{-1})	52	10.07 ± 0.16
Plasma glucose (mg %)	52	74.5 ± 0.77
Alkaline phosphatase (U l^{-1})	52	168.4 ± 3.65
Aminotransferase (ST ml^{-1})	52	53.3 ± 1.13
GSH-Px (U ml^{-1} cells at 30°C)	52	31.1 ± 1.05
Plasma Ca (m moles l^{-1})	50	2.48 ± 0.03
Plasma Mg (m moles l^{-1})	50	1.01 ± 0.01
Plasma Cu (μ moles l^{-1})	51	12.60 ± 0.23

(b) Mean concentrations of the various blood parameters determined for the nine cattle in Experiment 2 (Chapter 7) over the whole trial (±S.E.)

(i) Co supplemented (5 cattle)

Blood parameter	No. of samples	Trial mean
Haemoglobin (g 100 ml^{-1})	54	9.86 ± 0.20
Plasma glucose (mg %)	54	88.2 ± 1.84
Alkaline phosphatase (U l^{-1})	53	315.0 ±10.9
Aminotransferase (ST ml^{-1})	54	53.8 ± 2.27
GSH-Px (U ml^{-1} cells at 30°C)	54	45.1 ± 1.30
Plasma Ca (m moles l^{-1})	54	2.53 ± 0.04
Plasma Mg (m moles l^{-1})	54	0.90 ± 0.01
Plasma Cu (μ moles l^{-1})	54	13.09 ± 0.34
Urea (m mol l^{-1})	50	3.2 ± 0.16

(ii) Co depleted (4 cattle)

Blood parameter	No. of samples	Trial mean
Haemoglobin (g 100 ml^{-1})	44	10.31 ± 0.21
Plasma glucose (mg %)	44	88.3 ± 1.82
Alkaline phosphatase (U l^{-1})	42	312.4 ±17.2
Aminotransferase (ST ml^{-1})	44	51.7 ± 1.27
GSH-Px (U ml^{-1} cells at 30°C)	44	39.8 ± 1.60
Plasma Ca (m moles l^{-1})	44	2.53 ± 0.04
Plasma Mg (m moles l^{-1})	44	0.91 ± 0.02
Plasma Cu (μ moles l^{-1})	44	12.46 ± 0.39
Urea (m mol l^{-1})	40	3.4 ± 0.16

(c) Mean concentrations of the varous blood parameters determined for the ten cattle in Experiment 3 (Chapter 7) over the whole trial (± S.E.)

Blood parameters	No. of samples	Trial mean
Haemoglobin (g 100 ml^{-1})	20	11.17 ± 0.18
GSH-Px (U ml^{-1} cells at 30°C)	20	35.1 ± 1.41
Plasma Ca (m moles l^{-1})	20	2.78 ± 0.11
Plasma Mg (m moles l^{-1})	20	0.89 ± 0.03
Plasma Cu (μ moles l^{-1})	20	15.21 ± 0.52
Urea (m mol l^{-1})	20	3.9 ± 0.27

APPENDIX V Dietary analysis of feedingstuffs used in the cattle trials and the rations fed as described in Chapter 7

(a) Dietary analysis of the feedingstuffs used in the cattle trials (Chapter 7) and used for ration formulation

	Hay	Micronised maize	Prairie meal	Urea
DM g kg^{-1}	872	865	901	990
Metabolizable energy MJ kg^{-1}	7.5	14.2	14.0	0
Crude protein g kg^{-1}	72	94	524	2300
Ca g kg^{-1}	5.0	0.2	0.3	-
P g kg^{-1}	1.9	1.5	2.0	-
Na g kg^{-1}	1.2	0.06	0.26	-
K g kg^{-1}	-	4.44	0.75	-
Mg g kg^{-1}	1.8	0.6	0.6	-
S g kg^{-1}	1.37	1.13	7.59	-

(b) Daily rations fed to each calf (Chapter 7)

(i) Experiment 1

Approximate liveweight (kg)	Daily liveweight gain (kg)	Hay (kg)	Micronised maize (kg)	Prairie meal (kg)
100	0.7	1.5	1.1	0.3
150	0.7	2.7	1.1	0.3
200	0.7	3	1.5	0.4
225	0.7	3.3	1.6	0.4
250	0.7	3.7	1.7	0.4
275	0.7	3.7	2.0	0.4
300	0.7	3.7	2.1	0.4
325	0.7	3.7	2.4	0.4
350	0.7	3.7	2.5	0.5
350	1	4.2	3.1	0.9
375	1	4.2	3.5	0.9
400	1	4.2	3.6	0.9
425	1	4.2	3.8	0.9
450	1	4.2	4.0	1.0

Plus 50-100 g Dicalcium phosphate and 7 g salt

(ii) Experiments 2 and 3

All formulated to give a daily liveweight gain = 0.7 kg

Approximate liveweight (kg)	Hay (kg)	Micronised maize (kg)	Prairie meal (kg)	Urea (kg)
100	2-2.5	1.1	0.4	-
125	2.5	1.3	0.4	-
150	2.5	1.4	0.5	-
175	2.5	1.5	0.5	-
200	2.5	1.7	0.5	-
225	2.5	2.6	-	0.08
250	5.0	2.7	-	0.08
275	5.0	2.9	-	0.08
300	5.0	3.0	-	0.09
325	5.0	3.3	-	0.09

Plus 50-70 g Dicalcium phosphate and 2-3 g salt

APPENDIX VI Mean liveweights, haemoglobin, plasma Mg and Cu concentrations for the lambs used in the trace element supplemented anthelmintic evaluation trial (Chapter 8)

(a) Mean (n=20) liveweights (kg) for lambs at each sampling date in the trace element supplemented anthelmintic evaluation trial Chapter 8 (± S.E.)

(i) Site 1 (Grainston Farm)

Date	Control	Panacur + Cosecure	Panacur	Panacur SC
9/9/85	34.3 ± 0.86	35.0 ± 1.11	36.3 ± 0.97	34.9 ± 0.94
7/10/85	35.6 ± 0.75	35.6 ± 0.97	36.9 ± 0.97	36.2 ± 0.96
5/11/85	37.1 ± 0.76	36.2 ± 0.73	38.6 ± 0.85	37.3 ± 0.89
2/12/85	38.8 ± 0.72	38.3 ± 0.81	41.1 ± 1.03	39.3 ± 1.00
9/1/86	35.7 ± 0.73	34.7 ± 0.75	37.1 ± 0.72	36.4 ± 0.75

(ii) Site 2 (Orchardton Farm)

Date	Panacur	Panacur SC	Panacur + Vitamin B_{12} injection	Panacur + Co drench
1/10/85	42.3 ± 0.51	41.2 ± 0.99	42.9 ± 0.70	41.8 ± 0.79
29/10/85	43.2 ± 0.63	41.9 ± 0.95	43.1 ± 0.71	43.1 ± 0.72
25/11/85	47.6 ± 0.75	46.6 ± 0.70	46.9 ± 0.66	47.0 ± 0.59
17/12/85	47.3 ± 0.67	45.9 ± 0.76	46.7 ± 0.67	47.1 ± 0.56

(b) Mean concentrations of blood parameters for each group of lambs (n=20) used in the trace element supplemented anthelmintic evaluation trial (Chapter 8) over the whole trial period (± S.E.)

(i) Site 1 (Grainston Farm) - mean of 5 sampling dates

Blood parameter	Control	Panacur + Cosecure	Panacur	Panacur SC
Haemoglobin (g 100 ml^{-1})	12.4 ± 0.11	12.8 ± 0.11	12.5 ± 0.12	12.4 ± 0.10
Plasma Mg (m moles l^{-1})	1.07 ± 0.01	1.06 ± 0.01	1.03 ± 0.01	1.05 ± 0.01
Plasma Cu (μ moles l^{-1})	13.2 ± 0.33	15.6 ± 0.29	13.6 ± 0.44	14.1 ± 0.45

(ii) Site 2 (Orchardton Farm) - mean of 4 sampling dates

Blood parameter	Panacur	Panacur SC	Panacur + vitamin B_{12} injecton	Panacur + Co drench
Haemoglobin (g 100 ml^{-1})	12.4 ± 0.16	12.5 ± 0.17	12.7 ± 0.17	13.6 ± 0.16
Plasma Mg (m mole l^{-1})	1.03 ± 0.01	1.04 ± 0.01	1.05 ± 0.01	1.10 ± 0.01
Plasma Cu (μ moles l^{-1})	14.3 ± 0.56	16.4 ± 0.66	16.9 ± 0.78	16.8 ± 0.66

APPENDIX VII Yearly mean concentrations of the random blood parameters measured for the sheep in the experiment described in Chapter 9 (± S.E.)

(a) 1986

Blood parameters	Ewes		Lambs		
	Grazing untreated pasture	Grazing Co treated pasture	Grazing untreated pasture	Grazing Co treated pasture	Monthly vitamin B_{12} injection
Haemoglobin (g 100 ml^{-1})	11.9 ± 0.37	11.4 ± 0.35	11.5 ± 0.42	11.9 ± 0.26	12.1 ± 0.38
Plasma Mg (m moles l^{-1})	0.97 ± 0.04	0.92 ± 0.03	0.98 ± 0.04	0.95 ± 0.02	0.92 ± 0.03
Plasma Cu (μ moles l^{-1})	13.8 ± 0.7	15.6 ± 0.9	16.4 ± 1.3	14.9 ± 0.9	17.4 ± 1.5
GSH-Px (U ml^{-1} cells at 30°C)	43.8 ± 6.4	44.3 ± 5.2	51.5 ± 5.3	39.7 ± 4.1	45.9 ± 5.2

(b) 1987

Blood parameters	Ewes		Lambs		
	Grazing untreated pasture	Grazing Co treated pasture	Grazing untreated pasture	Grazing Co treated pasture	Slow release bolus
Haemoglobin (g 100 ml^{-1})	10.0 ± 0.77	9.7 ± 0.61	10.4 ± 1.05	10.3 ± 0.70	10.8 ± 1.57
Plasma Mg (m moles l^{-1})	0.95 ± 0.05	0.92 ± 0.05	0.95 ± 0.03	0.99 ± 0.03	0.96 ± 0.05
Plasma Cu (μ moles l^{-1})	15.2 ± 0.9	15.3 ± 0.8	15.9 ± 1.2	14.7 ± 0.8	14.9 ± 2.4
GSH-Px (U ml^{-1} cells at 30°C)	36.5 ± 8.0	38.3 ± 2.5	46.2 ± 6.4	48.3 ± 5.3	50.0 ± 13.8

N.B. Each taken as a mean of 5 random samples from each group at 2 sampling dates