ASPECTS OF THE COPPER-MOLYBDENUM-SULPHUR INTERACTIONS IN SHEEP

JANNES BERNARDUS JANSEN VAN RYSSEN

B.Sc.(Agric.) Pretoria, M.Sc.(Agric.) Natal

Submitted in partial fulfilment of
the requirement for the degree
of

DOCTOR OF PHILOSOPHY

in the

Department of Animal Science and Poultry Science
Faculty of Agriculture
University of Natal
Pietermaritzburg

December, 1979

I hereby certify that this research is the result of my own investigation.

J.B. Jansen van Ryssen

ACKNOWLEDGEMENTS

I wish to express with pleasure my appreciation to the following persons for their assistance in the preparation of this thesis :

Professor W.J. Stielau, Head of the Department of Animal Science and Poultry Science for invaluable assistance and advice as supervisor of this project.

Dr J.P. Kitching of Epol Feed Company for the examination of the carcasses and for advice and assistance during this investigation.

Mr B.D. Thring for assistance both at the experimental farm and in the laboratory throughout the investigation.

The following persons for laboratory assistance at various stages of the investigation : Miss F. Arangi, Miss A. Pienaar, Mrs P.Lamb, Mrs J.A. Holmes, Mrs R. Hill and Mr Z. Sibisi.

Mr P. Channon of the Cedara Research Institute, Mr F. Dekker of Allerton Veterinary Laboratories and Miss S. van Buuren of Edendale Hospital Laboratories for analyses performed by them.

Messrs William Mkize and Funani Ndhlovu for care and maintenance of the experimental animals.

Dr W.S. Botha, Department of Histopathology, Onderstepoort for examination and evaluation of histological preparations.

Mesdames S. Westwood, M.A. Kuyper and A. Putter for assistance during the preparation of this manuscript.

My wife, Irma, for her patience and assistance at all times - and my parents, for their encouragement throughout my academic career.

ABBREVIATIONS

The chemical symbols for elements were used throughout this manuscript, eg.

Cu - copper,
Mo - molybdenum
S - sulphur

NRC - National Research Council
DM - dry matter
IU - international units
PCV - packed cell volume
Hb - haemoglobin
GOT - glutamic-oxaloacetic transaminase
GPT - glutamic-pyruvic transaminase
LDH - lactic dehydrogenase
TCA - trichloroacetic acid
SE - standard error
SD - standard deviation
r - correlation
C_{IN}, C_{UR}, C_{CR} - clearances of inulin, urea and creatinine respectively
L. dorsi - longissimus dorsi muscle

CONTENTS

CHAPTER PAGE

T A B L E S

F I G U R E S

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

INTRODUCTION

In a world of growing food shortage the ruminant has a useful place because of its ability to convert nutrients otherwise inedible or unacceptable to the human, into highly nutritious products. This fact is well established in the utilization of roughage by ruminants who act not only as converters but also as harvesters of roughage inaccessible to man and his tools. Through industrialization and intensification of animal production, products become available, sometimes in such quantities as to cause environmental pollution (Smith, 1973; Blair, 1975). An example of this is the accumulation of excreta, as from feedyards or commercial poultry production. Smith (1973) stated that animal recycling systems for utilizing undigested feed nutrients and by-products of digestion and metabolism could place animals in an improved competitive position for supplying an even greater part of the world's future protein needs. Other available by-products suitable for ruminant conversion to human food include crop residues and by-products in the sugar, beer and plant oil industries.

It is most unlikely that such by-products can supply all the essential nutrients needed by the animal in a balanced form. Deficiencies and excesses of both essential and non-essential compounds can be expected if these by-products alone are fed. Blair (1975) indicated that the Animal Scientists could help to solve the problem of noxious industrial waste, by developing ways of processing such waste products and rendering them safe for inclusion in animal feeds.

Environmental or man-made imbalances of trace elements can cause a significant reduction of animal performance. This results in substantial economic loss and indirectly in a poorer nutritional status of human populations (Mertz, 1976). There is usually a rather wide range between the minimum dietary requirements of nutrients and maximum safe levels. However, all chemical agents, including essential elements are toxic in excessive amount (Mertz, 1976; Luckey & Venugopal, 1977; Neathery & Miller, 1977). Luckey & Venugopal, (1977) suggested that the secret of survival is not to absolutely prohibit the use of potentially toxic chemicals but to utilize each one rationally. Homeostatic mechanisms in the body can control tissue levels of essential elements with varying degrees of effectiveness. The body also has some protection from the inorganic forms of elements through the low absorption of these elements from the digestive tract (Neathery & Miller, 1977).

Many incidences of over-exposure to trace minerals are reported in the literature; those commonly condemned publicly include overdosages of the elements F, Cd, Pb, Se or As, even though some of them may be essential nutrients (Frost, 1979). The present study was prompted by local reports of Cu toxicity in sheep who are fed poultry manure. Since poultry manure is potentially an excellent source of non-protein nitrogen for livestock, its safe utilization should ideally be explored.

In South Africa poultry manure can be sold as an animal food only if it complies with certain nutrient and hygienic specifications. To satisfy the hygienic requirements it has to be processed, eg. by artificial drying. This substantially increases the price of the product (Denny, 1977). Consequently poultry manure is commonly purchased as fertilizer and then used as an animal feed. As part of an effort to ascertain the nutritional value of poultry manure and to gather data on its Cu content, poultry manure samples were collected from various sources in South Africa and subjected to detailed chemical analysis.

The use of Mo for protecting sheep against Cu toxicosis is well established (Dick, 1954). However, the precise relationship between Cu and Mo in the diet of ruminants has not yet been completely elucidated. Therefore, a series of trials was initiated in which the effects of various dietary levels of Cu, Mo or S on Cu and Mo metabolism were tested. It was further decided to expose sheep to high levels of Cu intake in order to simulate conditions of high Cu contaminations, as observed in broiler litter and possible other cases of Cu pollution of diets.

REVIEW OF LITERATURE

The metabolism of Cu in the body is characterised by its complexity. According to Abdellatif (1968) practically no other trace element exceeds Cu in the diversity of the factors which govern its absorption, excretion and utilization. This is clearly demonstrated by Suttle (1974a) who pointed out that diets of similar Cu content may produce clinical symptoms in sheep of either deficiency or toxicity. The predominant reason for this complexity is the effect of other substances, mainly other minerals, on the Cu metabolism in the animal. It has been shown that minerals such as Mo, S, Ca, Zn, Fe, Mn, Cd, Hg, Ag, Pb and Se can all influence Cu metabolism, either directly or by affecting other minerals antagonistic to Cu in the body of the ruminant (Dick, 1954; Tillman, 1966; Hill & Matrone, 1970; Awad, Ahmed, Lotfi & Fahmy, 1973; Bremner & Davies, 1973; Davies, 1974; Underwood, 1977). Consequently, no precise requirement of Cu by the ruminant can be assigned and a study of Cu metabolism cannot be done without considering the factors interacting with Cu (McDonald, 1970).

Dick (1954) managed to keep sheep in a positive Cu balance at Cu intakes as low as 1,0 mg/day. However, an intake of 0,5 mg Mo/day changed this positive retention into a negative Cu balance. Suttle, Field & Barlow (1970) under their experimental conditions calculated

the minimum Cu requirement to be 2 mg/sheep/day, while Suttle & Field (1974) estimated an endogenous Cu loss in ewes of 0,23 mg/day. Under so-called "natural conditions" the gross Cu requirement of sheep was calculated to be 3,85 mg/sheep/day or 7 mg/kg dry feed (Murty, 1957), 4,4 mg Cu/sheep/day (Abdellatif, 1968) and 4,1 mg Cu/sheep/day (Suttle, 1974a). The NRC standards of feed requirements for sheep (1975) accepted a level of 5 mg Cu/kg dry feed as being adequate for sheep. However, many cases of Cu deficiency disorders in cattle and sheep are quoted where the dietary Cu intakes of the animals appeared to be adequate (Mills, 1954; Russell & Duncan, 1956; Underwood, 1977). On the other hand, Cu toxicity symptoms have been reported in sheep receiving rations containing 7 to 11 mg Cu/kg DM (Todd, 1969). The NRC standards for sheep (1975) considered a Cu concentration of above 8 mg/kg DM as potentially toxic to sheep.

When administered in high doses, Cu can be an acute poison (Theiler, 1912; Ishmael, Gopinath & Howell, 1971a; Case, 1974). More commonly recorded though is the problem of the so-called "chronic Cu toxicity," characterised by a period of passive accumulation of Cu in the tissues during which time the animal exhibits no symptoms of toxicity. This is followed by an acute stage, referred to as the haemolytic crisis (Van Adrichem, 1965; Todd, 1969; Harker, 1976). The accumulation of Cu in the liver can continue for periods of a few weeks up to several years before the haemolytic crisis is experienced (Marston & Lee, 1948; MacPherson & Hemingway, 1965).

Of farm animal species, the sheep is the most susceptible to Cu toxicity (Todd, 1969; Ammerman, 1970; Underwood, 1977). Most cases of death due to high Cu intakes have been reported in mature sheep (Edgar, Hindmarsh, Keast & Rose, 1941; Todd, 1969; Bath, 1979). Although occasional reports of Cu toxicity in mature cattle have been reported (Todd & Gracey, 1959), it is generally accepted that cattle are far more resistant to Cu toxicosis than are sheep (Cunningham & Hogan, 1959; Todd, 1969; Underwood, 1977).

It was demonstrated by Bremner & Dalgarno (1973) that the availability of dietary Cu to preweaned calves was 50%. In the case of lambs Suttle (1975a) found it to be an even higher 71% but that this level dropped to 10,8% five days after weaning. This confirms the reports that young calves are almost as susceptible to Cu toxicity as are sheep (Shand & Lewis, 1957; Todd, 1969). Differences in susceptibility to Cu toxicity have been observed between sheep breeds, with British breeds being more susceptible than the Merino (Edgar et al., 1941; Marston & Lee, 1948).

The liver has been shown to be the almost exclusive site of Cu accumulation in the body (Dick, 1954), thus Cu concentrations here have proved to be a good indicator of the Cu status of the body (MacPherson & Hemingway, 1965; Ross, 1966). Although the retention of dietary Cu in the liver is low, usually less than 5% of total Cu intake, the accumulation was found to be linearly related to Cu intake (Dick, 1954; Hemingway & MacPherson, 1967). The length of this passive stage of Cu accumulation in the liver will depend on the level of Cu taken in, the period of its consumption and the presence of factors which interfere with Cu utilization in the sheep.

The haemolytic crisis stage of Cu toxicity has been variously observed at Cu concentrations ranging from 1100 to 6530 mg Cu/kg dry liver (Ogilvie, 1954; Clegg, 1956; Pearson, 1956; Bracewell, 1958; Gracey & Todd, 1960: Hill & Williams, 1965; MacPherson & Hemingway, 1965; Ishmael, Gopinath & Howell, 1971b). Dalgarno & Mills (1975) stated that they had observed deaths due to Cu toxicity at Cu levels in the liver of 700 mg/kg DM. They pointed out that significant risk may exist when hepatic Cu exceeds 1000 mg/kg DM. Case (1974) and Harker (1976) considered Cu levels of 500 mg and 600 mg/kg dry liver respectively, indicative of potential toxicosis.

Any stress on the sheep such as starvation, change in environment, transporting or excessive handling may bring about the haemolytic crisis (Todd & Thompson, 1963; Ross, 1964, 1966; Adamson, Valks, Appleton & Shaw, 1969; Bath, 1979). Usually only a small proportion of a flock succumbs to the disease. Bracewell (1958) reported

24 such deaths out of 720 ewes; Pierson & Aanes (1958) 90 out of 1500 ewes; Kowalczyk, Pope, Berger & Muggenburg (1964) 9 out of 300 sheep and Adamson et al. (1969) 3 out of 170 sheep. The wide variation in liver Cu concentration of sheep having the same Cu intake can probably explain this low proportion of deaths observed (Todd, Gracey & Thompson, 1962; Todd, 1969).

Typical symptoms observed in sheep during the haemolytic crisis include the following : the animals refuse to eat, are reluctant to move, become dull, the mucous membranes appear jaundiced, the urine is red-brown and the faeces brown, soft and sticky (Eden, 1940; Bracewell, 1958; Pierson & Aanes, 1958; Van Adrichem, 1965) A post-mortem reveals the carcass to be jaundiced with the fat and skin a deep yellow; the liver is enlarged, yellow and friable; the kidneys are enlarged through engorgement with blood and have a black metallic sheen very typical of Cu toxicity. Also the spleen is enlarged and the bladder contains dark coloured urine. Quite frequently the animals which died of Cu toxicity were otherwise in excellent nutritional condition (Ogilvie, 1954; Clegg, 1956; Pearson, 1956; Bracewell, 1958; Barden & Robertson, 1962; Adamson et al., 1969; Todd, 1969).

Marked histological changes have been observed in the liver and kidneys of sheep which had experienced a haemolytic crisis (Pearson, 1956; Bracewell, 1958; Ishmael et al., 1971b; Gopinath, Hall & Howell, 1974). The most characteristic biochemical change during the haemolytic crisis was elevated renal Cu levels (Todd et al., 1962; Ross, 1964), ranging from 100 to 500 mg Cu/kg DM at the crisis, as compared to normal kidney Cu levels of below 30 mg/kg DM (Ogilvie, 1954; Gracey & Todd, 1960; Todd et al., 1962; Ishmael, Gopinath & Howell, 1972). Blood and plasma Cu concentrations, haemoglobin and methaemoglobin levels showed steep increases during the crisis though haemoglobin and methaemoglobin levels decreased after 2 to 3 days (Marston & Lee, 1948; Gracey & Todd, 1960; Todd & Thompson, 1963; MacPherson & Hemingway, 1969; Ishmael et al., 1972). The glutathione levels in blood dropped to less than 10% of the pre-crisis levels (Todd & Thompson, 1963).

Dramatic increases in the levels of the serum enzymes GOT, GPT, LDH and arginase during the haemolytic crisis were reported by Todd & Thompson (1963), Ross, (1964), Van Adrichem (1965) Ross (1966), Hogan, Money & Blayney (1968) and MacPherson & Hemingway (1969). In fact, the GOT, LDH and arginase levels in the serum started to increase about six weeks before the onset of the crisis and have been used successfully as indicators of an approaching crisis (Van Adrichem, 1965; Ross, 1966).

Death may occur within hours after the appearance of the first symptoms of the haemolytic crisis (Edgar et al., 1941; Clegg, 1956). More commonly though, the illness results in death two to four days after the onset of the crisis (Bracewell, 1958; Todd, 1969). Some sheep, however, may survive one or even more crises in succession (Marston & Lee, 1948).

Occurrence of Cu toxicity

a) Natural conditions

In Australia a disease in sheep, Toxaemic jaundice, was proved to be due to chronic Cu toxicity. One form was the result of high Cu intakes through plants containing high concentrations of Cu. Another form was observed in sheep consuming plants containing normal levels of Cu, but very low levels of Mo. A third form, hepatogenous chronic Cu toxicity was the result of the consumption of plants containing hepato-toxic alkaloids, which damage the liver cells, leading to an excessive accumulation of Cu in these cells (Russel & Duncan, 1956; Todd, 1969; McDonald, 1970; Underwood, 1977).

Post-mortem symptoms of the disease, enzootic icterus ("geelsiekte") prevalent in the Karoo region of South Africa, were found to be very similar to those of Cu toxicity after the haemolytic crisis (De Kock, 1928; Brown, 1968). Cu concentrations of between 20 and 1400 mg/kg DM were measured in the livers of healthy sheep from this area

(Erasmus, 1970). However, Brown had previously (1968) concluded, after extensive studies, that no finality with regard to the role of Cu in this disease had been reached. In a review and summary of the problem Bath (1979) pointed out that enzootic icterus is in fact a form of chronic Cu toxicity and affected sheep reacted favourably to Mo treatment. Bath (1979) showed that although the Cu content of the herbage in the Karoo is fairly low, the Mo levels are exceptionally low (<1 mg/kg DM). Critically high liver Cu levels in sheep are normally only observed at a relatively old age. Any further stress, however, such as a drought (especially if combined with poor veld management practices) other diseases (such as "Geel-dikkop") or transportation of sheep, can trigger off the haemolytic crisis.

b) Pig feeds and slurry

Incidences of Cu toxicity have been reported in sheep and cattle fed on pig rations containing high levels of $CuSO_4$, a growth stimulant for pigs (Pearson, 1956; Todd & Gracey, 1959). Where hay from pastures previously sprayed with pig slurry rich in Cu, has been fed to sheep or where the sheep have grazed such pastures directly, deaths due to Cu toxicity have been known to occur (Loosmore, 1969; Van Ulsen, 1972). Cu at levels per kilogram DM of 643 to 1575 mg (Batey, Berryman & Line, 1972), 737 mg (Dalgarno & Mills, 1975), 22 to 636 mg (Pearce, 1975) and 325 mg (Kneale & Smith, 1977) were measured in pig slurry intended for application to pastures. The contamination of the herbage with Cu due to slurry adhering to the leaves after application (Batey et al., 1972; Dalgarno & Mills, 1975; Suttle & Price, 1976; Kneale & Smith, 1977) or from soil dust during harvesting (Dalgarno & Mills, 1975) resulted in high Cu consumptions by the animal. This, rather than an increase in the Cu content of the herbage itself is apparently the main source of the Cu consumed (Batey et al., 1972; Kneale & Smith, 1977).

c) Poultry manure

A high level of $CuSO_4$ is sometimes included in broiler diets to act as a growth stimulant (Smith, 1969) or as an antifungal agent

(Suttle, Munro & Field, 1978). Exceptionally high levels of Cu have been observed in the excreta of birds receiving additional $CuSO_4$, eg. 293 to 368 mg/kg air dry litter (Fisher, Wise & Filmer, 1972) and over 600 mg Cu/kg fresh litter (Fisher, Laursen-Jones, Hill & Hardy, 1973). Cases of mortality in sheep due to Cu toxicity after consumption of rations containing poultry manure high in Cu were reported by Fontenot, Webb, Libke & Buehler (1971) and Webb, Phillips, Libke, Harmon & Fontenot (1973).

d) Intensive rearing of sheep

The occurrence of Cu toxicity where sheep are fed concentrated rations has been reported and described as one of the major hazards in intensive rearing of sheep (Wensvoort & Hoskam, 1962; Koopman, 1963; Ross, 1966; Todd, 1969, 1972; Bremner, Young & Mills, 1976; Harker, 1976). Hogan et al. (1968) observed the death of ewes after 15 months on rations containing less than 9 mg Cu/kg DM; Adamson et al. (1969) in sheep on rations containing less than 20 mg Cu/kg DM and Todd (1969) in lambs at Cu intakes of 6 to 11 mg/kg DM. Bracewell (1958) reported 24 deaths out of 720 ewes fed a ration containing 43 mg Cu/kg DM.

The inadvertent inclusion of Cu, eg. through mineral supplements, anthelmintics or contamination in the pelleting of rations, can increase the Cu content of the ration (Todd, 1969). Harker (1976) pointed out that a Cu content of less than 12 mg Cu/kg DM is rarely attained in concentrate mixtures unless special precautions are taken in ration formulation. Protein concentrates are usually relatively high in Cu (Van der Berg & Van der Schee, 1973). Todd (1972) found low levels of Cu in barley and oatmeal; these feeds also contained exceptionally low levels of Mo. Low Mo and also low S levels in some concentrates have been suggested as important contributing factors to Cu toxicity observed in sheep on high concentrate rations (Suttle, 1974c; Thornton, 1974).

e) Incidental contact

Deaths have occurred in sheep which have inadvertently come into contact with copper containing compounds such as in pastures which have been treated with molluscicides for the control of liver fluke (Gracey & Todd, 1960) or which are in fallout zones near copper smelters (Case, 1974) or which are interplanted in orchards or vineyards where copper fungicides are used (Lafenetre, Monteil and Galtier, 1935; Ogilvie, 1954).

Cases of chronic Cu toxicity have also been reported where sheep consumed mineral supplement mixtures high in Cu (Clegg, 1956; Pearson, 1956; Kowalczyk, Pope & Sorensen, 1962) and in calves receiving copper-supplemented milk substitutes (Shand & Lewis, 1957; Dirksen & Hofmann, 1974).

McDonald (1970) mentioned that in certain areas of Australia the inclusion of $CuSO_4$ in fertiliser to prevent Cu deficiency in pastures has been taken to such a point that Cu toxicity in grazing stock has now become a risk factor.

Control of Cu toxicosis

a) Prevention

The most obvious approach in minimizing the risk of Cu toxicosis in sheep would be to keep Cu intakes as low as possible. This can be achieved by preventing sheep from consuming Cu-containing feeds such as contaminated orchard grass, pig feeds and mineral licks that are high in Cu, etc. Through control and pasture management sheep can be prevented from grazing pastures contaminated with pig slurry rich in Cu; cattle can be grazed under such conditions instead of sheep (Price & Suttle, 1975). To compile a concentrate ration low in Cu is difficult (Adamson et al., 1969; Harker, 1976) though Van der Berg & Van der Schee (1973) maintained that it is possible to produce a concentrate mixture of approximately 10 mg Cu/kg feed when the ingredients are properly chosen.

Where Cu has accumulated in the livers, the control and prevention of stress becomes important. Conditions such as insufficient feed, poor pasture management, the occurrence of other diseases or the transporting of sheep must be avoided to prevent the onset of the haemolytic crisis (Bath, 1979).

The prevention of the accumulation of Cu in the liver through management is not always possible and other prophylactic measures have to be relied upon. These measures are usually based on factors antagonistic to Cu in the body.

b) Factors interfering with Cu metabolism

The complexity of Cu metabolism in the body is mainly the result of factors directly interfering with Cu metabolism or antagonistic to those factors interfering with Cu. These interactions may occur at any one (or more) of the essential loci, eg. during gastro-intestinal or cellular absorption, in transport proteins or storage compounds such as cellular structural proteins. Excessive concentrations of one element within the cell may also prevent the effective utilization of another element in essential biochemical processes (Davies, 1974).

Tillman (1966) summarized the interactions involved in Cu metabolism as follows :

a) Cu is required for the efficient metabolism of Fe;
b) Cd and Ag increase the severity of Cu deficiency;
c) high dietary Zn reduces liver stores of Fe and Cu while low Zn favours excessive storage of Fe and Cu; an excess of Cu causes low storage of Zn ;
d) Mo limits the Cu storage in the presence of adequate sulphate.

In order to explain some of the interactions in the body, Hill & Matrone (1970) proposed that "those elements whose physical and chemical properties are similar will act antagonistically to each other biologically". The mutual interactions occurring in biological systems between Zn^{2+}, Cu^{+}, Cd^{2+} and Hg^{2+} illustrate this hypothesis (Hill & Matrone, 1970; Bremner & Davies, 1973; Davies, 1974). However, there are biological antagonisms involved in Cu metabolism which do not fit this chemical scheme, eg. the effect of Mo plus S on Cu or that of Ca on Cu, the so-called non-competitive interactions (Davies, 1974).

Examples of interactions have been observed in practice or under experimental conditions. Additional Zn and Fe were, for instance, required in pig rations high in $CuSO_4$ to prevent deficiencies of these minerals due to the presence of the Cu (Suttle & Mills, 1966). Reduced hepatic Cu concentrations have been observed with high intakes of Fe (Abdellatif, 1968; Standish, Ammerman, Simpson, Neal & Palmer, 1969) and Ca (Kirchgessner & Graßmann, 1970).

Various workers have reported reduced hepatic Cu concentrations in sheep and pigs when the protein levels of their rations were increased. Also differences in Cu retention were recorded when different sources of protein were fed (MacPherson & Hemingway, 1965; Combs, Ammerman, Shirley & Wallace, 1966; Goodrich & Tillman, 1966; O'Donovan, Spillane & O'Grady, 1966). However, Todd (1969) pointed out that different levels of Mo and S in the diets could explain some of the differences observed. In raising the level of protein in a ration the S intake can normally be expected to rise because of the increased intake of the S-amino-acids.

c) Molybdenum and sulphur

The most widely used method to control Cu toxicosis in sheep is the feeding of Mo plus S. Comprehensive studies in Australia on this problem of Cu toxicity in sheep culminated in the publication by Dick (1954) which indisputably established proof of the role of Mo in the presence of SO_4^{2-} on Cu metabolism in sheep. Since this report by Dick (1954), various recommendations for treating and preventing Cu toxicosis have been published. A summary by Pope (1975) of these, is given in Table 1.1.

The wide variation in levels of Mo and SO_4^{2-} recommended, is obvious from the table. Under experimental conditions where intakes were controlled, Wynne & McClymont (1956) reported the

Table 1.1 Methods of treating or preventing Cu toxicosis in sheep (after Pope, 1975)

Treatment or prevention	Period
Drenching	
100 mg NH_4Mo* + 1 g Na_2SO_4 in 20 cm^3 H_2O/day/lamb	13 weeks
50 - 500 mg NH_4Mo* + 0,3 - 1 g thiosulphate/sheep/day	3 weeks
Fertilizing	
Mo (38 g/hectare) + superphosphate	
In salt lick	
84,8 kg salt + 635 kg gypsum + (0,45 kg Na Mo* dissolved in 7,57 dm^3 H_2O sprayed on)	free choice
Feeding	
150 g NH_4Mo* + 2,3 kg Na_2SO_4 (to provide 100 mg NH_4Mo* and approx. 15 g Na_2SO_4/head) + 18,9 dm^3 H_2O, sprayed on hay	3 weeks
100 mg NH_4Mo* + 1 g Na_2SO_4/0,45 kg of pellets/lamb/day	3 weeks
38 mg Mo + 5,2 g SO_4^{2-}/day supplied in pelleted rations	15 months

* Molybdate

development of hypocuprosis expressed as low hepatic Cu levels, hypocupraemia, hypochromatrichia and dystrophic wool. This was observed at a dietary Cu level of 5,2 mg together with 5,14 mg Mo and 0,4% SO_4^{2-} supplemented to the feed. Marcilese, Ammerman, Valsecchi, Dunavant & Davis (1969) observed at an intake of 13 mg Cu, 50 mg Mo and 0,4% SO_4^{2-} daily that liver Cu concentrations in sheep dropped from 1151 to 301 mg/kg DM, accompanied by an impairment of caeruloplasmin synthesis. Van der Berg & Van der Schee (1973) reported that the liver Cu concentration was 372 mg/kg DM in sheep receiving 25,4 mg Cu and 22 mg Mo as compared to liver Cu levels of 1559 mg/kg DM in the control without Mo. Similarly, Harker (1976) reported a 40,1% reduction in liver Cu when 7,7 mg Mo + 0,42% SO_4^{2-} was supplied to sheep receiving 24,4 mg Cu/day.

These few examples demonstrate not only the value but also the major hazard in the use of Mo as a prophylactic measure against Cu toxicosis. In sheep, molybdenosis is usually expressed in the form of hypocuprosis. In fact, Suttle & Field (1968) pointed out that the choice between molybdenosis or induced hypocuprosis as a description of the syndrome produced with Mo and SO_4^{2-} supplementation in sheep must remain an arbitrary one. Symptoms commonly ascribed to molybdenosis per se as reported in cattle, viz. diarrhoea, anorexia and loss in body mass, are usually not observed in sheep, even when grazing the same pasture (Cunningham & Hogan, 1959; Underwood, 1977; Ward, 1978). It is generally accepted that sheep are far more tolerant of high Mo intakes than are cattle (Bingley, 1974; Case, 1974; Underwood, 1976).

Mo metabolism and toxicity in ruminants have been reviewed recently (Underwood, 1976, 1977; Ward, 1978). Relevant sections of this vast and complex field will be discussed in subsequent chapters. Although the value of Mo + S in the control of Cu toxicity is well established, caution is still necessary in their use, because of the danger of induced Cu deficiency. Mills (1974), Bremner et al.(1976) and Harker (1976) emphasized that Mo may be included in the diet only

with due regard to the daily Cu intake. Harker (1976) pointed out that Mo cannot be recommended for general use until further research has established safe levels of Mo with regard to actual Cu content of the feed. The problem as defined by Huisingh, Gomez & Matrone (1973) is that the effect of any one of the elements Cu, Mo or S is dependent on both the previous and present dietary levels of the other two elements. Combined with this is the problem of establishing the Cu status of the animal (Bremner & Marshall, 1974). Although the liver Cu level is a reliable indication of the Cu status of the sheep (MacPherson & Hemingway, 1965; Ross, 1966) the animal has to be slaughtered to obtain this information, or liver biopsies have to be done. Hogan, Money & Walker (1971) observed a uniform distribution of Cu in the livers of mature sheep, while Barden & Robertson (1962) had previously measured fairly large variations in Cu concentration at different sites on the liver containing high levels of Cu. A non-uniform distribution of liver Cu would reduce the value of liver biopsies. However, biopsy samples should still provide a reasonably good indication of the Cu level of the liver at high Cu concentrations.

Plasma Cu levels in sheep usually remain within the normal range of 0,7 to 1,2 mg Cu/dm^3 (MacPherson, Brown & Hemingway, 1964; Claypool, Adams, Pendell, Hartmann & Bone, 1975). Even at high Cu intakes, these levels were maintained up to the onset of the haemolytic crisis stage of Cu toxicity. Therefore, except under conditions of Cu deficiency where low plasma Cu concentrations could be associated with low liver Cu levels (MacPherson et al., 1964; Claypool et al., 1975), plasma Cu concentrations are of no value as indicators of the Cu status of the animal.

By following changes in serum enzyme levels Van Adrichem (1965) and Ross (1966) were able to estimate the onset of tissue breakdown due to Cu accumulation in the body. Mo treatment could be

started with the use of this information. However, Todd et al. (1962) observed that once the clinical symptoms of the haemolytic crisis occurred, the illness was so acute that recovery after treatment with Mo did not always occur.

Miltimore & Mason (1971) concluded from their observations and analyses of feeds that the risk of Mo toxicity in cattle was high if the diets contained a Cu : Mo ratio of less than two. Case (1974) quoted a Cu : Mo ratio of 7 : 1 as the safest level for cattle rations while Alloway (1973) and Pope (1975) considered Cu : Mo ratios of 5 : 1 as physiologically desirable for sheep.

d) Mechanisms of Cu, Mo and S interactions.

The mechanisms of the metabolic interactions between Cu, Mo and S are largely speculative, especially at blood and tissue levels. Various proposals and possible mechanisms have been put forward (Huisingh et al., 1973; Suttle, 1974b; Dick, Dewey & Gawthorne, 1975; Huisingh & Matrone, 1976). In general, the evidence suggests that interactions exist between Cu, Mo and S and also between Cu and S, between Mo and S and possibly between Cu and Mo. Dick (1954) concluded from his studies that the reaction between Cu and Mo was only possible in the presence of dietary SO_4^{2-}. Dick (1956) proposed the blocking of Cu transport across membranes by Mo and SO_4^{2-} as the mechanism involved. Under in vitro conditions Dowdy & Matrone (1968) observed the formation of an insoluble Cu - Mo complex similar to the compound lindgrenite. It was suggested that this compound is absorbed, transported and excreted as a unit from the body (Dowdy & Matrone, 1968; Huisingh et al., 1973). However, Suttle (1974b), Smith & Wright (1975a) and Huisingh & Matrone (1976) expressed doubts about the existence of such a complex under in vivo conditions.

Suttle (1974b) and Dick et al. (1975) suggested the formation of cupric thiomolybdate complexes in the body as one possible mechanism

of the Cu - Mo - S interaction. Dick et al. (1975) proposed the formation of thiomolybdates in the digestive tract between sulphide and molybdate. These are suggested to bind Cu to form insoluble cupric thiomolybdates, thus limiting the absorption of dietary Cu. Fisher & Clawson (1976) argued that the complex formed, should rather be cuprous ammonium thiomolybdate. Dick et al. (1975) proposed that the unbound thiomolybdate can be absorbed and mobilize tissue Cu in the body which could be excreted through the urine. This theory was supported by Gawthorne & Nader (1976) who observed the formation of di-,tri-, and tetrathiomolybdates in the rumen.

Elevated concentrations of plasma Cu have been observed at high dietary Mo intakes. This was attributed to an increase in so-called "direct reacting" Cu (DR Cu, i.e. plasma Cu soluble in diethyl dithiocarbamate) and to a fraction called "residual Cu" which is also TCA-insoluble (Smith, Field & Suttle, 1968; Suttle & Field, 1968; Bingley, 1974; Smith & Wright, 1975 a,b). This is accompanied by elevated kidney Cu levels and an increased urinary Cu excretion (Dick, 1956; Marcilese, Ammerman, Valsecchi, Dunavant & Davis, 1970). The existence of these foreign Cu-Mo complexes in the blood was described by Suttle (1974b) as being true systemic effects of the Cu - Mo - S interaction, though the relative importance of these systemic effects was considered as rather small (Suttle, 1975b). He also proved that organic S was as effective in the Cu - Mo interaction as inorganic S in the form of SO_4^{2-}.

The depressing effect of S on hepatic Cu content, independent of Mo, was observed by Wynne & McClymont (1956), Goodrich & Tillman (1966); Boyazoglu, Barrett & Du Toit (1972), Huisingh et al. (1973) and Suttle (1974c). Suttle (1974c) showed that organic and inorganic sources of S were equally effective in this interaction and claimed that those workers who did not observe a decrease in hepatic Cu content due to S, used less than 2 g S/kg feed. The formation of CuS in the digestive tract which is unavailable to the body, was suggested and proved to be the mechanism involved (Mills, 1960; Huisingh et al., 1973; Suttle, 1974 b,c).

Intravenously injected S did not exert any effect on Cu metabolism, with or without Mo (Marcilese et al., 1969), suggesting that this interaction occurs in the digestive tract. Suttle (1974c) stated that there was evidence that the critical site of CuS formation may not be the rumen but that it takes place lower down in the digestive tract. Huisingh & Matrone (1976) suggested that the formation of CuS was the more significant route of rendering Cu unavailable in the rumen than the formation of Cu - Mo compounds.

Two mechanisms of interaction between Mo and S have been reported. Huisingh et al. (1973) and Huisingh & Matrone (1976) pointed out that SO_4^{2-} and MoO_4^{2-} are both tetrahedral anions with the same charge. They proposed that both SO_4^{2-} and MoO_4^{2-} use the same carrier in an active tissue transport system, notably in the intestinal wall and the kidney tubules. The presence of SO_4^{2-} would reduce the absorption of Mo from the digestive tract, leading to a drop in the Mo becoming available to the body. Excess SO_4^{2-} may also prevent the resorption of Mo through the kidney tubules, resulting in higher Mo excretion rates in urine.

Evidence for these proposals is seen in the decreased Mo levels in tissues with the addition of S to rations (Dick, 1956; Cunningham, Hogan & Lawson, 1959; Vanderveen & Keener, 1964; Cook, Lesperance, Bohman & Jensen, 1966; Suttle, 1975b; Grace & Suttle, 1979) and increased urinary Mo excretions (Dick, 1956; Marcilese et al., 1970). Mason & Cardin (1977) showed, with the aid of in vitro segment incubation studies, that SO_4^{2-} can inhibit the uptake of MoO_4^{2-} in various segments of the small intestine of sheep.

Dietary Mo is also involved in the reduction of SO_4^{2-} in the rumen. A rapid reduction of SO_4^{2-} to S^{2-} was observed to take place in the rumen. A proportion of this S^{2-} is absorbed through the rumen wall (Bray, 1969; Hume & Bird, 1970; Bryden & Bray, 1972). A sharp drop in S^{2-} production in the rumen was experienced in the presence of Mo, apparently because Mo inhibited the ATP-sulphurylase system

of rumen sulphate-reducing bacteria (Bryden & Bray, 1972; Gawthorne & Nader, 1976; Huisingh & Matrone, 1976). The addition of Mo to a diet would therefore increase the SO_4^{2-} concentration of the ingesta which can again inhibit Mo absorption from the digestive tract (Grace & Suttle, 1979). Another action of Mo is to inhibit the rate of absorption of S^{2-} through the rumen wall (Gawthorne & Nader, 1976). The role of Mo in this process is apparently of a catalytic nature in which small quantities of Mo can block this pathway of S^{2-} absorption (Gawthorne & Nader, 1976). This will influence the rate at which ruminal S^{2-} concentrations will decrease because of the inhibition of S^{2-} formation from SO_4^{2-}.

It is evident from the literature that quite a degree of uncertainty still exists regarding Cu metabolism in the interaction of Cu with Mo and S in the ruminant. This is to be expected, considering all the other factors which can interfere with Cu metabolism and the difficulty in monitoring the mineral status of the animal body. In many of the studies on the Cu - Mo - S interaction, effects are overshadowed by the symptoms of the induced Cu deficiency. Although a Cu deficiency may be expected under natural conditions, high levels of Cu intake by animals may occur, or high levels of Cu accumulation in body tissues may have been reached before any measures were taken to prevent Cu toxicity. The approach in the present series of trials was to follow the distribution of minerals in the body when Cu deficiency symptoms were not present. Semi-purified diets are usually used in trace mineral studies. This approach may be essential if consequences of interactions are investigated at low levels of mineral intake. However, nutrients present in normal livestock rations may be utilised differently from those in semi-purified diets. Practical farm rations were therefore used in these trials. The distribution of Zn and Fe in the body was also followed to obtain some information on these two minerals when the Cu and Mo interactions were investigated. Four trials were carried out in which different dietary levels of Cu, Mo or S were supplied to sheep in order to obtain information on the Cu and Mo metabolism and the Cu - Mo - S interaction in their bodies.

CHAPTER 2

MATERIALS AND METHODS

GENERAL PROCEDURES

ANIMALS AND MANAGEMENT

a) Experimental animals and terrain

South African Mutton Merinos, drawn from the flock of the Ukulinga Research Station, were used in Trials 1, 3 and 4. In Trial 2 Corriedales were used. The latter type had been obtained from a farm in East Griqualand some six months before the start of the trial. The sheep were grouped according to sex, where applicable, and such that all groups had the same numbers of sheep and approximately the same total mass. Treatments were randomly allocated to the groups. The sheep were identified with numbered ear tags, a different colour for each treatment. The treatment groups were kept at Ukulinga Research Station in concrete floored pens partially covered with a lean-to roof. Ample feeding space was provided to reduce competition between sheep, by allowing each sheep unrestricted access to feed.

b) Body mass and shearing

The body mass of each sheep was determined at the onset of the trials, one day before slaughtering and at various stages during the trials. On every occasion feed and water was withdrawn for 18 hours prior to recording the body mass. The sheep were shorn 2 to 3 weeks before the onset of each trial.

c) Dosing and inoculations

All sheep were dosed with anthelmintics before the onset of each trial. Faecal worm egg counts were done on random samples at various stages of the trials to monitor the level of parasitic infestation; no cases of parasitism were detected.

Before the onset of the trials the sheep were inoculated against black quarter, pulpy kidney, blue tongue and botulism.

FEEDS AND FEEDING PROCEDURES

a) Feeds, feed preparation and feeding routine

In Trial 1 the sheep received long Eragrostis curvula hay and in the remaining trials milled veld hay (predominantly Themeda triandra) was fed. Hay from a single cut each year was used during a given experiment. The daily hay allowance for a group was measured out and fed once a day in concrete feed troughs. Any left-overs were collected as necessary in order to determine total feed consumption. The hay was sampled regularly for DM determinations, the samples being ground in a Wiley mill and stored pending chemical analyses.

Additional Cu, Mo and S, according to treatments, was supplied in the form of cupric sulphate ($CuSO_4.5H_2O$), ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) and sodium sulphate (Na_2SO_4). The minerals were mixed, first in a small quantity of concentrates, using a dough mixer, then with the other ingredients of the concentrate mixtures in a 500 kg feed mixer. Samples were taken from every mixed batch for DM determination and further chemical analyses. Concentrate allowances for one or two weeks were measured out, from which the daily allowances per group were fed in PVC feed troughs. Concentrate consumption was always complete. Tap water was supplied ad libitum in PVC troughs during the first two trials and in concrete troughs during the last two trials.

All sheep received a compound mixed vitamin A,D and E injection shortly after the onset of each trial. In Trial 4 a second vitamin injection was given towards the end of the trial.

COLLECTION AND TREATMENT OF SAMPLES

a) Slaughtering and processing of organs and tissues

Sheep in the first three experiments were slaughtered at the Pietermaritzburg abattoir and those from Trial 4 at the Baynesfield Factory abattoir. Organs and tissues were collected as soon as possible after slaughter, placed in plastic bags, sealed and brought to the laboratory cold room (-20° C). The fresh mass of the organs was determined usually within 4 hours after slaughter and after removing any adhering tissue including the gall bladder and any blood or fluid from the organs. Organs and tissue for DM determinations were dried in a forced draft oven maintained at 80 to 100° C for 4 to 5 days. The dried samples were finely ground and stored for subsequent mineral analysis. Care was taken to ensure that the samples were representative of the organs and tissues concerned.

In the case of the liver, samples were taken randomly at various sites from the liver. In Trial 1 V-shaped cross-sectional kidney slices including both the cortex and the medulla were taken. Only kidney cortices were collected in Trials 2 and 3 while cortices and medullas were dried separately for analyses in Trial 4. Entire spleens and both testes, where available, were prepared for analyses. After removal of the trachea, a composite sample of lung tissue was taken and samples of ventricular cardiac muscle were collected after the removal of fat. A muscle sample of approximately 5 cm^3 was taken from the M. Longissimus dorsi in the area of the last rib, cleared from connective tissue and dried. In the case of the heart and muscle tissue, fat extraction with ether was necessary before analyses.

In Trial 4 samples from the livers (centre of right lobe) and the kidneys (cortex and medulla) were collected and placed in 10% buffered formalin for histological evaluations. Samples were about 1 cm^3 in size.

b) Blood

Jugular blood samples, collected in the morning prior to feeding, were taken at regular intervals during all trials. If whole blood or plasma samples were required, saturated sodium citrate (3 drops/ 10 cm^3 blood) was used as an anticoagulant. During blood collection in the kidney clearance tests (Trial 4) heparin was used as the anticoagulant.

c) Wool

Wool samples from the one side of each sheep were collected at the end of Trials 1 and 4. Dirty tips were cut off before the wool was washed and the fat extracted. During Trial 4 the clean, fat-free wool was teased, rinsed with distilled water and dried before analyses.

ANALYTICAL PROCEDURES

GENERAL

Glassware was acid-washed for mineral determinations and only distilled-deionised water was used in analyses. All reagents used were of Analar grade whenever possible.

DIGESTION OF SAMPLES FOR MINERAL DETERMINATIONS

a) Dry ashing

The ashing of samples in a muffle furnace was done overnight at 500° C. The ashed samples were dissolved in 25% of V/V HCl and transferred through filter paper into volumetric flasks.

b) Wet acid digestion

Wet ashing with concentrated acids was performed in 100 ml Erlenmeyer flasks on a sand bath. In the case of body tissues 0,5 cm^3 H_2SO_4, 2,5 cm^3 $HCCl_4$ and 5 cm^3 HNO_3, added separately, were used per 1 g of sample. The H_2SO_4 was omitted to prevent the formation of $CaSO_4$ when samples containing high levels of Ca were digested..
The samples were left in the acid mixture at least overnight. This allowed for a preliminary digestion in the cold, thus decreasing the occurrence of excessive frothing and the loss of samples when heated up. Complete acid digestion was found to be essential, particularly so in the Fe and Mo determinations. The digested samples were transferred with distilled-deionised water into volumetric flasks.

c) Dry versus wet ashing

Differences between mineral concentrations of duplicate samples tended to be greater in the dry ashing than in the wet ashing method, though results were comparable. Since more samples could be handled per batch in the wet than in the dry ashing method, wet acid digestion, therefore, came to be the method of choice subsequent to the analyses of the survey samples which were dry ashed. However, in the case of the hay samples low in Cu and Mo, larger samples per container could be more conveniently handled in the dry than in the wet ashing method and dry ashing was therefore used in most of the mineral analyses on hay.

ANALYTICAL METHODS

a) Feeds and body tissue

The Cu, Zn, Fe and Mn content of feed , poultry manure and body tissue samples as well as the Ca, K and Mg levels in the survey samples were determined on an Atomic Absorption Spectrophotometer (Varian Techtron, Model 1000).

The P and Na contents of the survey samples were determined by flame emission and N by means of the Kjeldahl method (AOAC, 1965). The Ca, P and N levels of the feeds from the trials were analysed on an Auto Analyzer (Technicon Auto Analyzer II).

The method of Blanchar, Rehm & Caldwell (1965) was used to determine the S content of the feeds.

The molybdenum-iron-thiocyanate method as described by Blamey (1971) was slightly modified for the Mo determination of feeds, tissues and plasma. These modifications consisted of :

1. Acid digested samples were used subsequent to the survey.
2. Ten cm^3 of the 6,5 N HCL · $FeCl_3$ reagents were added to all separating funnels.
3. Five cm^3 of a 1 : 1 iso-amyl alcohol : chloroform mixture were used as the extractant instead of iso-amyl alcohol and carbon tetrachloride.
4. By centrifuging the stannous chloride reagent, it was found to be unnecessary to centrifuge the extractant containing the coloured substance. The extractant was poured through medium to fast filter paper directly into the spectronic tubes.

With each batch of body tissue a mixed poultry liver sample was used as a reference sample. With the feeds, a mixed concentrate sample served the same purpose. The chemical composition of these samples was accurately known. Standard reference mineral solutions (G.D. Searle, S.A.) were used in atomic absorption measurements. Working concentrations of such solutions were prepared by diluting the standard solutions with 0,1 N HCl in distilled-deionised water.

b) Blood and urine

The Cu concentration in plasma and serum was measured by atomic absorption spectrophotometry on samples diluted with 8% butanol in a ratio of 1 : 2 (Suttle, 1974a).

Direct reacting Cu plus the standards were analysed according to the method described by Suttle & Field (1968).

Plasma Mo concentrations were determined according to the Blamey (1971) method, modified, as previously described.

The Ca, Mg, Zn, Fe and K levels in serum were measured by atomic absorption spectrophotometry.

The method described by Taussky & Shorr (1953) was used to determine the inorganic P concentration in serum.

Total serum protein and albumin, after precipitation of globulin, was estimated using the biuret method described by Cornelius & Karneko (1963).

Packed cell volume (PCV) was determined by a micro-haematocrit method, and a Coulter counter was used to obtain red blood cell counts. Blood haemoglobin levels were assessed by means of the "Biochemica" test combination of Boehringer Mannheim.

Boehringer Mannheim test combinations were also employed in the determination of serum glutamic oxalo-acetic transaminase (GOT), serum lactic dehydrogenase (LDH).

Plasma arginase was determined according to the method described by Schwartz (1971).

The cadmium sulphate filtrate method as described by Smith (1956) was used to determine inulin in plasma and urine.

The picric acid method based on the "Jaffe reaction" was used in the determination of creatinine in plasma and urine on an Auto Analyser (Technicon Auto Analyzer II).

The diacetyl-monoxime method as modified for an Auto Analyser was used to determine urea in plasma and urine (Technicon Auto Analyzer II).

HISTOPATHOLOGY

The routine methods of tissue preparation were carried out on tissue samples preserved in buffered formalin. Two staining techniques were used, viz. the Haematoxylin-Eosin and the "Periodic Acid Schiff" (PAS) methods (Humason, 1962).

KIDNEY CLEARANCE PROCEDURE

In general, the procedures as described by Smith (1956) and Owen (1975) were followed to determine the kidney clearances of inulin, endogenous creatinine and endogenous urea. One ewe at a time was taken and after body mass was determined, the ewe was sedated with a general tranquillizer (acetyl promazine) according to prescription. This was considered to be essential preparation in view of the fact that stress can initiate the haemolytic crisis stage of Cu toxicosis in sheep (Todd, 1969). No feed was permitted but access to water was allowed prior to the measurement of kidney clearances.

A Foley's balloon type catheter (12FG) was inserted into the bladder. A polythene catheter for blood collection was inserted in the right jugular vein, filled with heparin and secured to the skin. In the left jugular vein a polythene tube was introduced through a 15G needle. After the priming dose of inulin (40 cm^3 inulin solution of a 1,25 g inulin/50 cm^3 sterile saline) was introduced through this tube, it was connected to a Unita II continuous infusion apparatus (B. Braun - Melsungen). Inulin at a concentration of

3,75 g/500 cm^3 sterile saline was infused into the sheep at a constant rate of 1,5 to 2 cm^3/minute, depending on the size of the ewe. Urine collection started after at least 45 minutes of infusion, to allow the inulin concentration in the blood to stabilise.

Urine was collected for 20 to 30 minutes depending on urine flow. About 3 minutes before the end of the collection 10 or 20 cm^3 distilled water (depending on urine flow) was used to rinse the bladder. Blood was collected at the mid-point of each urine collection period and centrifuged immediately after collection. Four blood and urine collections were made per clearance and samples were analysed for inulin, creatinine and urea.

Calculation of renal clearance, eg. inulin :

$$C_{IN} = \frac{U_{IN} V}{P_{IN}}$$

C_{IN} = clearance of inulin

U_{IN} = urine concentration of inulin (mg/cm^3)

V = urine flow (cm^3/minute)

P_{IN} = plasma concentration of inulin (mg/cm^3) (Smith, 1956).

STATISTICAL ANALYSES

The F- and the Student's t-tests were used to calculate the significance of differences between treatments. Standard procedures to calculate regressions and correlations were employed (Rayner, 1967). In those instances where other statistical methods were used, the techniques applied are given in the relevant chapters.

CHAPTER 3

A SURVEY OF THE MINERAL CONTENT OF POULTRY MANURE FROM SOUTH AFRICAN SOURCES

INTRODUCTION

Poultry manure has been used successfully in South Africa as a substitute for more expensive protein sources in ruminant rations (Anonymous, 1960; Bishop, Wilke, Nash, Nell, MacDonald, Compaan, Grobler & Kingman, 1971; Van der Westhuizen & Hugo, 1972). However, little attention has been paid to the high mineral content of manure incorporated into ruminant rations, and cases of Cu toxicity in sheep have been reported in Natal due the the consumption of poultry manure containing high levels of Cu. In order to obtain information regarding the mineral content of the poultry manure in South Africa, poultry manure samples were collected from various poultry enterprises in the country.

PROCEDURE

On request by letter or through personal collection, representative samples of poultry manure were obtained from various poultry farms in South Africa. Details were requested for each sample regarding type of enterprise, housing and flooring, litter material used, period of accumulation, feed company supplying the ration, etc. This information was used in the classification of the samples into groups.

On arrival the samples were dried at 80° C, ground and stored, pending analysis. Chemical analyses using the methods described in Chapter 2 were done on these samples.

RESULTS AND DISCUSSION

It was possible to group the poultry manure samples into three general classes, viz. pure excreta from birds in batteries, excreta from broilers in deep litter systems and excreta from pullets and breeders in deep litter systems. The mineral content of the poultry manure, as presented in Table 3.1 varied widely within classes, and there were also distinct differences between classes. The largest differences occurred between battery manure and broiler deep litter. Although the average mineral levels in pullet/breeder deep litter samples were intermediate to those obtained for layers and broilers, the range of concentrations for the majority of minerals in the pullet/breeder deep litter samples was wider than for the other two types, indicating that the pullet/breeder samples were of more diverse origin.

The high ash and mineral content of the manure samples given in Table 3.1 clearly indicates that poultry manure can be an excellent source of minerals for livestock. However, the wide variations observed even within samples of similar origin would be a major limitation complicating the proper formulation of rations, unless each batch of manure is individually analysed. When the mineral content of the manure samples is compared to the mineral requirements of sheep (Table 3.2) as quoted by Pope (1975) it is clear that the inclusion of poultry manure in the rations of sheep can supply significant amounts of required minerals. None of the trace elements, except Cu, was present in dry poultry manure at levels higher than the toxic amounts quoted by Pope (1975). The average correlation for the three types of manure between ash and Ca was 0,74 and between ash and Fe 0,62. Most of the other minerals showed low but positive correlations with the total ash percentage.

Copper

The broiler manure could be divided into two groups: those samples with an average of 28 mg Cu/kg DM, ranging from 5 to 52 mg/kg and the remainder with an average of 424 mg Cu/kg DM ranging from 296 to

Table 3.1 Mineral and protein content of poultry manure on a dry matter basis (Survey results)

Nutrient	Pure battery manure (n = 8)			Broiler Deep Litter (n = 12)			Pullet & Breeder Deep Litter (n = 14)		
	average	± SE of mean	range	average	± SE of mean	range	average	± SE of mean	range
Calcium (%)	6,26[b]	1,76	4,14 - 8,41	1,83[b]	0,70	0,92 - 2,97	3,66[b]	2,11	0,94 - 7,62
Phosphorus (%)	1,84[a]	0,35	1,11 - 2,30	1,44[a]	0,39	0,51 - 1,89	1,62	0,22	1,25 - 2,08
Ca : P ratio (:1)	3,63	-	2,06 - 7,58	1,38	-	0,85 - 2,80	2,18	-	0,63 - 4,28
Magnesium (%)	0,75[b]	0,24	0,34 - 1,08	0,47[b]	0,09	0,36 - 0,70	0,60	0,18	0,34 - 0,94
Potassium (%)	1,71[b]	0,51	1,00 - 2,62	1,10[ba]	0,20	0,85 - 1,43	1,46[a]	0,37	1,14 - 2,66
Sodium (%)	0,35[a]	0,08	0,22 - 0,51	0,48[ad]	0,15	0,31 - 0,86	0,37[d]	0,10	0,16 - 0,55
Sulphur (%)	0,66[a]	0,18	0,47 - 1,08	0,60[ab]	0,08	0,55 - 0,81	0,51[ab]	0,12	0,30 - 0,85
Copper (mg/kg)	36[a]	9,04	23 - 53	192[ab]	214,9	5 - 570	47[b]	23,6	10 - 97
Zinc (mg/kg)	364[ad]	133,0	246 - 661	261[a]	63,4	170 - 361	264[d]	72,9	208 - 484
Manganese (mg/kg)	533[b]	74,8	455 - 697	418[b]	88,9	290 - 595	469	95,2	309 - 629
Molybdenum (mg/kg)	0,73	0,50	0,36 - 1,84	1,15	1,01	0,45 - 4,30	0,89	0,20	0,41 - 1,11
Iron (mg/kg)	1576[bc]	252	919 - 3259	700[b]	39	506 - 17603*	962[c]	121	452 - 1784
Ash (%)	30,8[b]	4,66	20,6 - 37,0	13,7[bd]	5,58	9,1 - 30,0	19,0[bd]	6,99	9,3 - 33,9
Crude protein (N x 6,25) (%)	22,8[c]	4,50	19,0 - 32,7	24,7[b]	3,06	19,5 - 31,1	16,9[bc]	3,77	9,9 - 26,1

* The value 17603 mg/kg Fe has been excluded from calculations.

For the same nutrient, values with the same superscripts a or d differ at $P<0,05$ and b or c differ at $P<0,01$ levels of significance.

Table 3.2 The minerals required by sheep (Pope, 1975)

Major elements		Trace elements		
Mineral	Requirements % of DM	Mineral	Requirements mg/kg DM	Toxic amount mg/kg DM
Na	0,04	Fe	30 - 50	Unknown
Ca	0,21 - 0,52	Cu	5	8+
P	0,16 - 0,37	Mo	0,5	5 - 20
Mg	0,04 - 0,08	Mn	20 - 40	Unknown
K	0,50	Zn	35 - 50	1000
S	0,14 - 0,26			

570 mg/kg. The high Cu levels in the latter group probably reflect the supplementation of $CuSO_4$ as a growth stimulant or its inclusion as an antifungal agent in broiler rations. Fontenot et al. (1971) reported sheep mortalities due to Cu toxicity from a ration containing 191 mg Cu/kg DM as a result of the inclusion of broiler litter high in Cu. At the level of 424 mg Cu/kg DM in broiler litter, rations containing even fairly small proportions of such manure, may well result in final Cu levels which could lead to Cu toxicity in sheep.

In South Africa a Cu concentration of 50 mg/kg manure is the maximum allowed for the registration of poultry manure as an animal feed (Denny, 1977). At this level of Cu, the inclusion of 10% of manure will be sufficient to supply the Cu requirements of sheep (Pope, 1975).

Suttle & Price (1976) established that the Cu in poultry waste was biologically as available to sheep as the Cu in $CuSO_4$. This is contrary to the claim by Lowman & Knight (1970). Suttle & Price (1976) suggested the inclusion of Cu antagonists in rations containing dried poultry waste, especially when rations contained cereals low in Mo. They based this recommendation on information gathered from Cu depletion-repletion trials, using low levels of Cu, small quantities of manure (5%) and semipurified diets. Whether any additional Cu antagonists are actually required at higher manure intakes and normal feed ingredients, is debatable because of the high concentration of Cu antagonists such as Fe, Zn, S and Ca already present in manure.

Copper antagonists

The S levels of the analysed samples were high, ranging from 0,30 to 1,08% on a DM basis. Suttle (1974c) suggested that impairment of Cu metabolism due to S could be expected if rations contained more than 0,2% S. The contribution of poultry manure to the S content of mixed rations should in most cases be sufficient to obtain S levels of well above 0,2% and interactions between S and Cu are therefore to be expected.

The Mo levels of the poultry manure samples were low except for one sample with a Mo content of 4,3 mg/kg DM. Even though Dick (1954) observed that Mo at levels of 0,5 mg/day influenced the Cu metabolism in sheep, the effect of such low levels of Mo in rations high in Cu content can be expected to be minimal.

The Fe content of the poultry manure samples was very high. Pure battery manure contained an average of 1576 mg Fe/kg DM. One sample with a Fe content of 1,7% was well above the level measured in the other samples and was therefore omitted from calculations. The Fe levels in the samples compared well with Fe concentrations given in the literature, eg. 630 mg/kg DM (Lowman & Knight, 1970), 2500 mg/kg DM (Jimenez, 1974) and 1660 mg/kg DM (Essig, 1975). The toxic level of Fe to sheep is unknown (Pope, 1975). At a level of 4 g FeS/day Dick (1954) observed liver Cu concentrations in sheep of 90 mg/kg DM as compared to 222 mg/kg for control sheep not fed FeS. This reduction could have been due to both the Fe and the S present in FeS. Abdellatif (1968) reported reduced liver Cu concentrations at Fe intakes of 2,6 and 5,2 g /sheep/day. Standish et al. (1969) observed decreased plasma and liver Cu concentrations in cattle at levels of Fe supplementation of 400 and 1600 mg/kg feed. At both these levels of Fe intake Standish et al. (1969) also observed decreased feed intakes and body mass gains. Lawlor, Smith & Beeson (1965) observed excessive diarrhoea in lambs consuming rations which contained 210 and 280 mg Fe/kg DM. Mineral imbalances at these levels of Fe intakes were suggested as a possible cause of the diarrhoea. Although the Fe levels used by Dick (1954) and by Abdellatif (1968) were exceptionally high, it seems that the normal levels of Fe in poultry manure may also reduce the availability of Cu to the animal.

The practical significance of the interactions between Cu, Zn and Fe has been demonstrated by Suttle & Mills (1966) in pigs receiving high levels of $CuSO_4$ as a growth stimulant. At levels of 250 and 425 mg Cu/kg feed, mild to severe symptoms of Cu toxicosis in the form of Zn and Fe deficiencies were experienced. With the addition of 150 mg Zn and 150 mg Fe/kg feed these Cu toxicity symptoms could

be overcome. Since Zn levels at 800 to 1000 mg/kg feed are safe for sheep the feeding of Zn as safe alternative to Mo has been suggested (Bremner & Davies, 1973; Mills, 1974). In 1954 Dick observed that 100 mg Zn/day significantly reduced the hepatic Cu content of sheep from 207 mg to 148 mg. Similarly, Bremner et al. (1976) found the addition of 220 to 420 mg Zn/kg feed to be an effective method of reducing the hepatic Cu retention in sheep. The presence of fairly high levels of Zn in poultry manure will therefore most likely influence Cu metabolism even when present in mixed rations.

The Ca and P levels in the survey samples corresponded well with levels reported by Blair (1974) and by Emerson (1975). The average P percentages in Table 3.1 were slightly lower than the minimum values required by the Department of Agricultural Technical Services in South Africa for registration of poultry manure as a livestock feed, viz. a minimum of 2,0% for cage birds and 1,5% for broilers (Denny, 1977). Jimenez (1974) quoted Ca retentions in sheep of 88% and P retentions of 66%, from poultry manure. Field, Munro & Suttle (1977) found the P in poultry manure to be readily available to sheep. However, Kirchgessner & Grassmann (1970) observed a decrease in hepatic Cu retention in cattle when Ca levels increased from 5 to 15 g Ca/kg feed. They ascribed this effect of Ca on Cu as due to a change in rumen pH at higher Ca intakes, making Cu less soluble. Increased levels of Cu excretion in the faeces with increased Ca intakes were also considered by Suttle & Field (1970) as evidence confirming that Ca had a negative effect on Cu retention in sheep. Dick (1954) observed reduced Cu retentions in the livers of sheep receiving Ca in the form of $CaCO_3$ (90 g/sheep/day) but not when dicalcic phosphate was fed. The amount of Ca contributed by poultry manure to a mixed ration will in most cases be more than the Ca requirements of sheep and may therefore also contribute to antagonistic actions to Cu.

Against the background reviewed it becomes clear that the composition of the overall ration may greatly modify the toxicity of a given mineral. Fox & Reynolds (1973) remarked that "an excess

intake of an essential nutrient over the usually recognized requirement may be protective against a toxic element". While this finding is comforting from the point of view of practical animal production, it complicates efforts aimed at elucidating relationships between individual dietary minerals. The high concentrations of those minerals in poultry manure, antagonistic to Cu metabolism in ruminants, should be able to offer some protection against Cu toxicity. The magnitude of such effects will be difficult to establish because the metabolism of these minerals may in turn be influenced by the other minerals present in manure.

On a related matter Field et al. (1977) reported some evidence of urolithiasis in the sheep consuming poultry manure. The incidence of urinary calculi in sheep tended to increase at high P intakes, depending on the Ca content of the diet (Pope, 1975). The type of poultry manure, its level of inclusion in rations and other ingredients in the basic diet all go to determine the extent to which urolithiases will be a problem in sheep consuming poultry manure.

CHAPTER 4

TRIAL 1: THE INFLUENCE OF MO AND S ON THE CU METABOLISM OF SHEEP RECEIVING HIGH LEVELS OF CU AND BROILER LITTER IN THEIR RATIONS

INTRODUCTION

broiler litter as an ingredient of rations may be potentially dangerous to sheep because of the high Cu content in litter from some broiler houses (Chapter 3). In order to utilize this source of N in sheep nutrition, the threat of Cu toxicity has to be contained. The combination of Mo plus S has long been used to control the accumulation of Cu in sheep livers (Dick, 1954). However, the danger of Mo toxicity exists, usually expressed in the form of an induced Cu deficiency, if Mo plus S are given without caution (Harker, 1976).

A trial was conducted to examine the influence of Mo and S on sheep receiving high levels of Cu in rations containing broiler litter.

PROCEDURE

Experimental animals, treatment and procedure

Forty S.A. Mutton Merino wethers, approximately two years of age were randomly allocated to five treatments, viz. a pre-experimental slaughter treatment (Pre-exp.), a control (Control), a high Cu (Cu), a high Cu plus high Mo (Cu + Mo) and a high Cu, high Mo and high S (Cu + Mo + S) group. The trial lasted 157 days.

The groups received long Eragrostis curvula hay and a basic concentrate mixture consisting of 57% broiler litter and 43% maize meal. The broiler litter with wood shavings as litter material, obtained

from a commercial source, was sieved and mixed with the maize meal. The experimental routine as described in Chapter 2 was followed.

At slaughter the livers, kidneys, spleens and muscle samples from the M. longissimus dorsi were collected and treated as described in Chapter 2. Wool samples were taken from the side of the sheep for chemical analyses at the end of the trial.

Chemical analyses were completed according to the methods described in Chapter 2. The data obtained comprised the following: the Cu, Fe, Zn, Mn, S, Mo, Ca, P and crude protein levels of the feeds; the Cu and Mo content of serum, liver and wool; the Cu content of kidneys (representative samples from both cortex and medulla), spleen and muscle; the Fe and Zn levels of the liver, and the PCV of whole blood.

RESULTS

Feed intake and composition of rations

The average hay intake was 607 g DM and the concentrate intake 654 g DM/sheep/day. The mean daily intake of broiler litter which contained 23,5 mg Cu/kg DM and 0,59% S on a DM basis, was 373 g or 29,6% of the daily mean DM consumption. The concentration of minerals in the total ration and the mineral intake/sheep/day are given in Table 4.1. Minerals potentially antagonistic to Cu in the body, viz. Zn, Fe and Ca, are included in Table 4.1. The relatively high concentration of these minerals in the rations was partly due to the high content of these minerals in poultry manure. The average crude protein intake was 193 g /sheep/day.

Clinical condition

No clinical signs of Cu toxicity were observed during the trial, nor did any other problems related to the feeding of the minerals or broiler litter become apparent. All sheep increased in body mass

Table 4.1 Total mineral intakes/sheep/day and average concentration of minerals in experimental rations (DM basis)

Treatments	Cu mg	Mo mg	Zn mg	Fe mg	Mn mg	S g	Ca g	P g
	Daily intake of minerals/sheep							
Control	17,0	0,48	83	438	242	3,3	10,6	7,9
Cu	75,4	0,53	80	434	254	3,4	10,8	8,2
Cu + Mo	73,4	57,6	96	457	271	3,4	11,7	8,7
Cu + Mo + S	70,1	60,9	82	410	243	6,1	10,9	8,0
	Concentration of minerals							
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	%	%
Control	13,5	0,38	66	348	192	0,26	0,84	0,63
Cu	59,8	0,42	64	344	202	0,27	0,85	0,65
Cu + Mo	58,2	45,72	76	362	215	0,27	0,92	0,69
Cu + Mo + S	55,6	48,31	65	324	192	0,48	0,86	0,63

without any differences between the groups (Table 4.2). At slaughter the mass of the fresh organs and the DM content of these organs did not differ significantly between treatments. None of the organs from individual sheep within treatments showed oedemic or other abnormal conditions.

Minerals in body tissues and wool

The addition of Cu to the ration resulted in a highly significant increase in the Cu content of livers (Table 4.3). Within the Cu group, liver Cu concentrations ranged from 505 to 1388 mg/kg DM. The addition of Mo to the Cu supplemented ration reduced liver Cu levels by approximately 40% and the combination of Mo + S added to Cu supplemented rations further decreased liver Cu levels to values not statistically different from those of the controls.

The addition of Mo with or without S dramatically increased kidney Cu levels. Significantly higher Cu levels were also measured in the spleens of those groups receiving Mo or Mo + S as compared to the other treatments.

The concentrations of Mo, Zn and Fe in the liver and Cu and Mo in clean, fat-free wool are given in Table 4.4. The addition of Mo to the rations resulted in significant increases in the liver Mo concentrations above those treatments without Mo. Additional S in the Cu + Mo + S group did not change the Mo concentration of the liver significantly from the Cu + Mo level. Cu, Mo or S had no significant influence on the Zn and Fe levels of the liver. None of the differences in Cu or Mo levels of wool between treatments were statistically significant, mainly because of big variations in the Cu and Mo levels within treatments.

Blood analyses

No significant differences in PCV were observed between treatments

Table 4.2 Body mass and mass increase, fresh mass and dry matter percentage of organs at end of trial

	Treatments			
	Control	Cu	Cu + Mo	Cu & Mo + S
Final body mass (kg)	43,2	42,7	42,4	41,9
Mass increase (kg)	9,1	8,2	6,8	7,4
Liver :				
fresh mass (g)	511	513	490	497
dry matter (%) *	29,3 ± 0,27	29,7 ± 0,35	30,0 ± 0,46	29,9 ± 0,31
Spleen :				
fresh mass (g)	68,1	67,9	70,0	64,4
dry matter (%) *	22,0 ± 0,08	21,9 ± 0,09	22,2 ± 0,14	22,0 ± 0,15
Kidneys :				
fresh mass (g)	111,5	104,9	101,8	101,6
dry matter (%) *	20,2 ± 0,15	20,2 ± 0,21	20,4 ± 0,28	20,5 ± 0,34

* Means ± SE

Table 4.3 The influence of Cu, Mo and S intakes on the accumulation of Cu in the body tissues of sheep (± SE of mean)

		Treatments**			
	Pre-exp.*	Control	Cu	Cu + Mo	Cu + Mo + S
Liver :					
Concentration (mg/kg DM)	176	$282^{a} \pm 31$	$878^{b} \pm 94$	$517^{c} \pm 89$	$401^{ac} \pm 98$
Total Cu content (mg)	23,5	43^{a}	124^{b}	74^{d}	62^{ad}
Cu retention as % of Cu intake		0,73	0,85	0,44	0,35
Kidneys :					
Concentration (mg/kg DM)	18,9	$22^{a} \pm 2,03$	$21^{a} \pm 0,84$	$161^{b} \pm 14,98$	$157^{b} \pm 18,89$
Total Cu content (mg)		$0,48^{a}$	$0,44^{a}$	$3,36^{b}$	$3,27^{b}$
Spleen :					
Concentration (mg/kg DM)	9,8	$4,3^{a} \pm 0,54$	$5,4^{2} \pm 1,30$	$11,2^{b} \pm 1,51$	$11,5^{b} \pm 1,62$
Total Cu content (mg)		$0,064^{a}$	$0,080^{a}$	$0,174^{b}$	$0,167^{b}$
Muscle (L. dorsi) : (fat-free)					
Concentration (mg/kg DM)	7,3	$6,7 \pm 1,40$	$5,3 \pm 0,17$	$7,7 \pm 0,54$	$7,3 \pm 0,87$

* Minerals in tissues of pre-experimental slaughter group calculated using estimated tissue DM values. Data not included in statistical analyses.

** Different superscripts designate differences between treatment averages: a - b and a - c at $P<0,01$; a - d at $P<0,05$ levels of significance

Table 4.4 The influence of Cu, Mo and S intakes on the concentration of Mo, Fe and Zn in the liver and Cu and Mo in clean, fat-free wool (± SE of mean)

	Treatments*			
	Control	Cu	Cu + Mo	Cu + Mo + S
Liver :				
Mo (mg/kg DM)	$4,0^{a} \pm 0,23$	$4,3^{a} \pm 0,44$	$35,6^{b} \pm 3,67$	$39,5^{b} \pm 4,39$
Zn (mg/kg DM)	115 ± 5,7	112 ± 5,9	114 ± 7,8	111 ± 4,8
Fe (mg/kg DM)	240 ± 20,9	220 ± 20,5	195 ± 18,0	208 ± 12,9
Wool : (fat-free)				
Cu (mg/kg DM)	5,89 ± 0,41	6,13 ± 0,39	4,90 ± 0,45	5,11 ± 0,70
Mo (mg/kg DM)	0,87 ± 0,54	0,65 ± 0,36	2,05 ± 0,13	1,28 ± 0,40

* Different superscripts designate differences between treatment averages at $P < 0,01$ level of significance.

(Table 4.5), though the PCV values increased in all groups from an average of 28,7% at the onset of the trial to an average of 30,6% at the end of the trial. The average serum Cu and Mo levels did not change markedly over the last three months of the trial (Figure 4.1 and 4.2 respectively). Serum Cu levels during the last three months of the trial were significantly higher ($P<0,01$) in the Mo supplemented groups than in the other two treatments. Since no analyses were carried out in December, there is uncertainty about the nature of the rise in serum Cu concentration during the first two months of the trial. The average serum Cu and Mo concentrations are presented in Table 4.6. The two Mo treatments caused a significant increase in serum Cu and Mo levels above the treatments without Mo. Additional S at the high Mo intake reduced serum Mo levels significantly below the Mo levels where Mo was given without S.

DISCUSSION

The ability of Mo to decrease the Cu content of the liver by some 40% has been previously reported by other workers, including Dick (1954). Harker (1976) observed a 41% decrease in liver Cu due to Mo supplementation while Wynne & McClymont (1956) had previously reported a reduction of close to 100% in liver Cu content, with concomitant signs of hypocuprosis. The extent and risk of this depression depends on the Cu status of the liver, the duration of Mo feeding, and the dietary levels of Cu and Mo during this period (Suttle, 1975b).

Additional S caused a further drop in hepatic Cu content though the effect was relatively small as compared to the effect of Mo only. Dick (1954) demonstrated that S was essential in the Cu - Mo interaction and that an increase in dietary S depresses hepatic Cu levels even further. It is evident from the present trial that the S in the basic diet was biologically available and that it was present at a sufficiently high level to potentiate the action of Mo on Cu

Table 4.5 Influence of Cu, Mo and S intakes on packed cell volume (PCV) in blood during the different months of Trial 1

Treatment	PCV (%)			
	Jan.	Febr.	March	April
Control	28,6	27,5	31,3	30,4
Cu	28,6	27,7	30,5	29,4
Cu + Mo	27,8	28,1	30,8	30,8
Cu + Mo + S	29,9	29,2	31,6	31,8

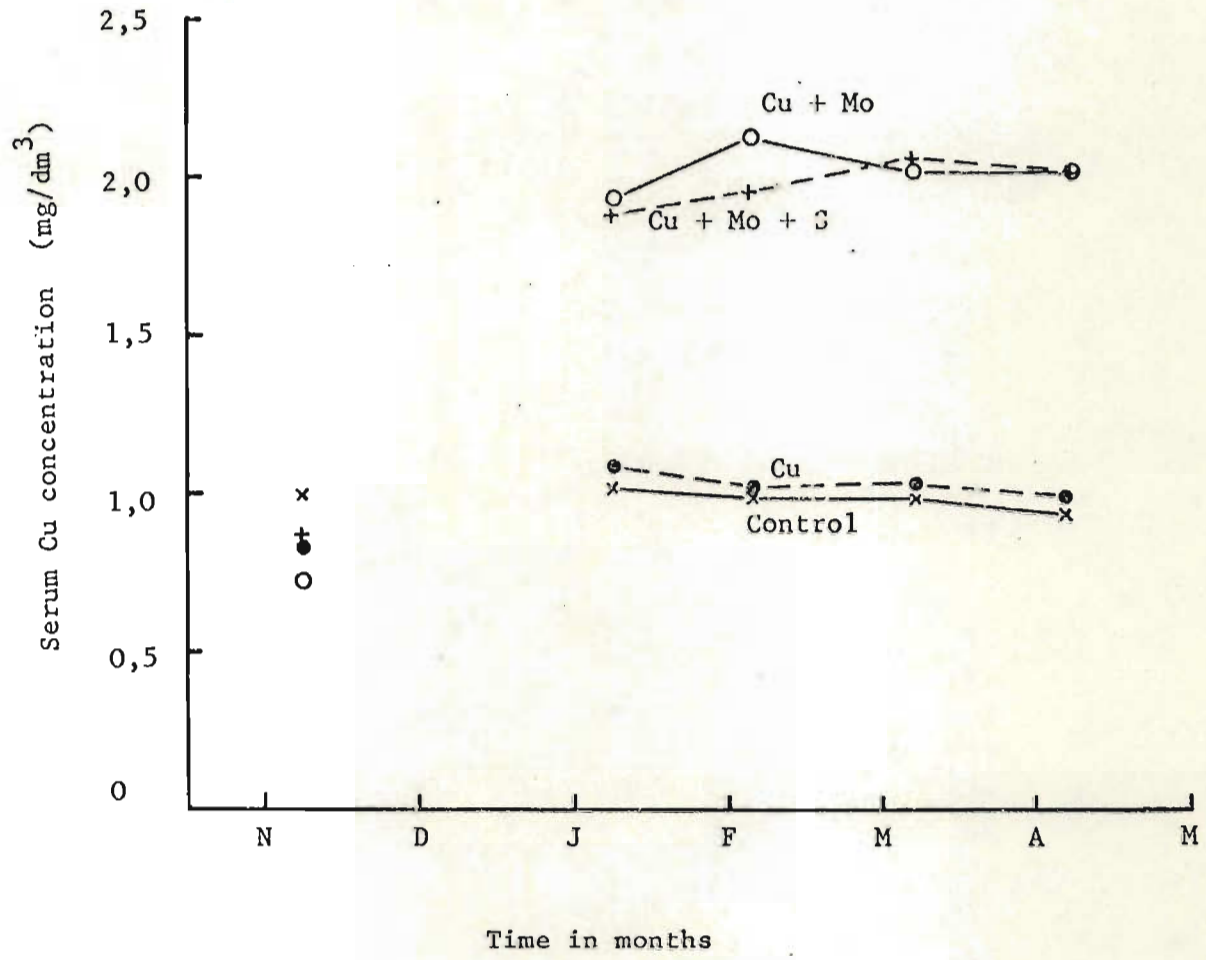

Fig. 4.1 Concentration of Cu in the serum of sheep fed different levels of dietary Cu, Mo and S

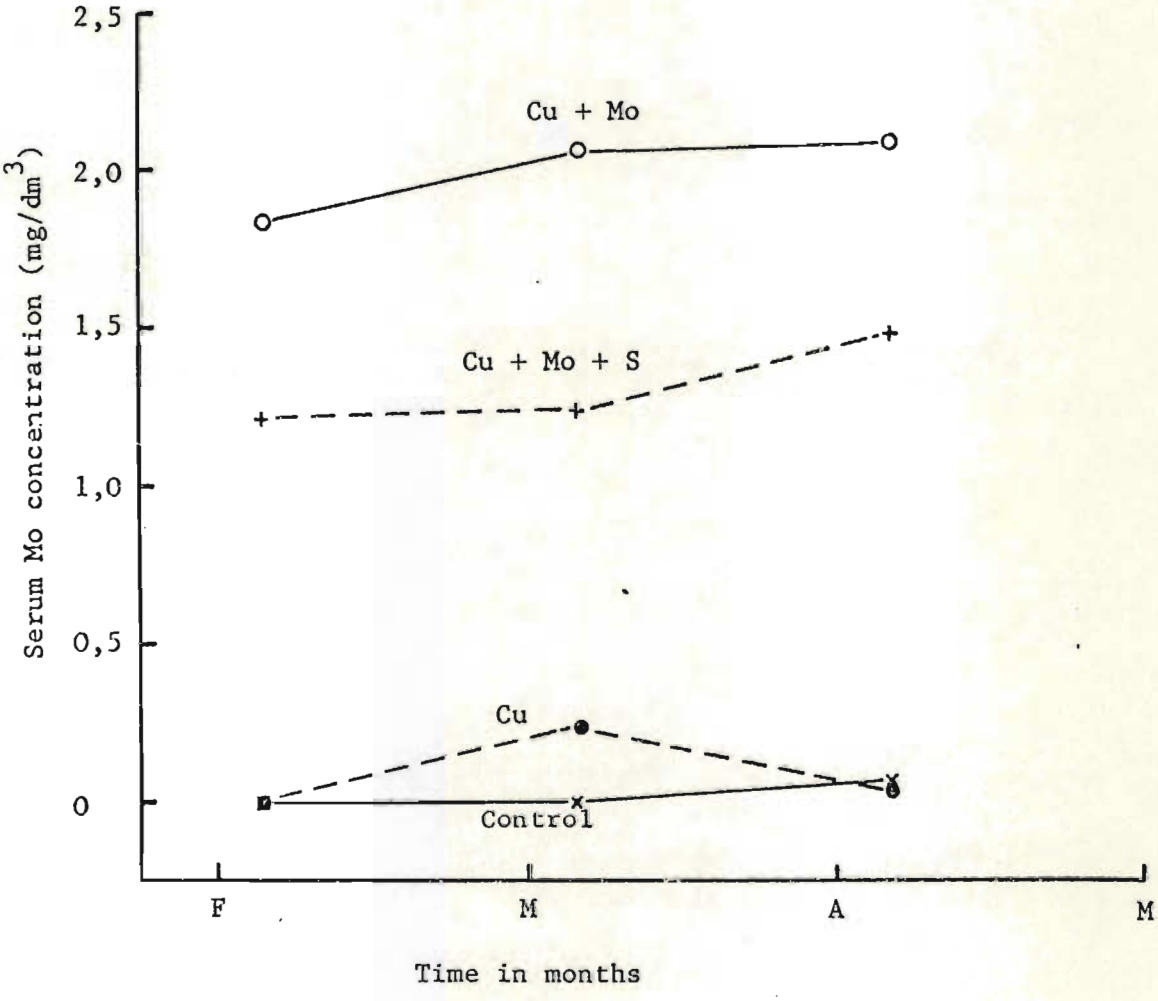

Fig. 4.2 Concentration of Mo in the serum of sheep fed different levels of dietary Cu, Mo and S

Table 4.6 The influence of Cu, Mo and S intake on the Cu and Mo levels in blood serum

	Treatments*			
	Control	Cu	Cu + Mo	Cu + Mo + S
Pre-experimental collection				
Cu (mg/dm^3)	1,00	0,83	0,74	0,85
Averages of last four months				
Cu (mg/dm^3)	$1,00^a$	$1,04^a$	$2,04^b$	$1,94^b$
Mo (mg/dm^3)	$0,04^a$	$0,10^a$	$2,00^b$	$1,30^c$

* Different superscripts designate differences between treatment averages at $P < 0,01$ level of significance.

metabolism. The high S content in poultry manure contributed substantially to the S content in the basic ration used. The presence of a fair proportion of poultry manure in a ration serves to obviate the need for supplying additional S with Mo when the latter is used as a prophylactic against Cu toxicity.

In both treatments where no additional Mo was supplied, very low hepatic Cu retentions (0,73 and 0,85%) were observed. The percentages of Cu retained in the livers were also very similar in the two treatments despite widely different levels of Cu intakes (13,5 mg versus 59,8 mg Cu/sheep/day). In the absence of high levels of substances in the rations antagonistic to Cu metabolism in the body, dietary Cu was found to be retained in livers at a rate of 3,3 to 5% (Dick, 1954), 2,96 to 3,01% (MacPherson & Hemingway, 1965) and 3,2 to 8,3% (Suttle, Munro & Field, 1978). The relatively high level of S present in the basic ration (0,26%) may have contributed to the low hepatic Cu retention observed in the trial. A depressing effect of S *per se* on Cu availability was observed by Wynne & McClymont (1956), Goodrich & Tillman (1966) and Boyazoglu *et al.* (1972). The intake of other minerals potentially antagonistic to Cu in the ruminant body, such as Fe (Standish *et al.*, 1969), Zn (Bremner *et al.*, 1976) and Ca (Kirchgessner & Grassmann 1970) were also well above the requirements of sheep (Pope, 1975) and may have contributed to the low absorption and retention of Cu in this trial. To what extent the breed of sheep used, contributed to the low Cu retention is not clear. However, breed differences in susceptibility to Cu toxicity have been observed (Edgar *et al.*, 1941).

The finding of a decrease in the Mo concentration of serum and of wool with the addition of S to the high Mo treatment is in agreement with the observations by Dick (1956), Suttle (1975b) and Bremner (1976). However, the liver Mo levels remained high in the treatments receiving additional Mo and did not decrease with the addition of S to the diet. This result, with respect to liver, is contrary to the expected response as observed by Dick (1956) and Huber, Price & Engel (1971) and no explanation for this observation is apparent.

The dramatic increase in serum Cu concentration at high dietary Mo levels is consistent with results reported by Dick (1956), Suttle & Field (1968), Bingley (1974) and Smith & Wright (1975a). This increase was attributed to an increase in the "Direct Reacting" Cu fraction in plasma and the occurrence of a so-called "residual" Cu fraction in plasma (Suttle & Field, 1968; Smith & Wright, 1975 a, b). Elevated plasma Cu levels due to dietary Mo were also accompanied by increased Cu concentrations in the kidneys and higher Cu urinary excretion rates (Dick, 1956; Smith et al., 1968; Marcilese et al., 1970). The formation of stable unavailable Cu - Mo - S compounds has been suggested as the cause of these elevated plasma and kidney Cu levels (Suttle, 1974b ; Smith & Wright, 1975 a, b).

A dramatic increase in renal Cu levels accompanied by kidney damage has been observed during the haemolytic crisis stage of Cu toxicity (Todd et al., 1962 ; Ross, 1964 ; MacPherson & Hemingway, 1969). Even though Van Adrichem (1965) and Ross (1966) treated sheep with Mo plus S at the first sign of an approaching crisis, viz. elevated GOT levels in plasma, the kidney Cu levels of the sheep remained well above normal at slaughter, and signs of kidney damage were observed by Van Adrichem (1965). The kidney damage was attributed to the Mo + S treatment and Van Adrichem (1965) warned against the risk of kidney damage due to the prolonged use of Mo + S in the treatment of Cu toxicity. This aspect was, inter alia, investigated in Trial 4.

The problem of urolithiasis in sheep was observed at high P intakes when the Ca : P ratio in the ration was narrow (Pope, 1975). The high percentages of P in the basic ration during the present trial and the narrow Ca : P ratio indicate that this could become a problem in rations containing high levels of broiler litter.

CHAPTER 5

TRIAL 2 : THE EFFECT OF VARIOUS LEVELS OF DIETARY CU AND MO ON CU AND MO METABOLISM

INTRODUCTION

The depressing effect of Mo and S on hepatic Cu content as observed during Trial 1, is well established and amply confirmed in the literature. However, recommendations regarding levels of Mo and S to be used in the prevention of Cu toxicosis vary tremendously (Pope, 1975). In order to obtain further information on relative concentrations of Cu and Mo to be used for this purpose, the following trial was carried out in which different levels of Cu and Mo were fed, while the daily S intakes were held constant.

PROCEDURE

Experimental animals, treatments and procedures

In this trial 56 Corriedale wethers between 1½ and 2 years of age were divided into seven groups of eight sheep each. The treatments were :

Treatment P - a pre-experimental slaughter group ;
Treatment 1 - high Cu, high Mo for 92 days ;
Treatment 2 - medium Cu, high Mo for 92 days ;
Treatment 3 - low Cu, high Mo for 92 days ;
Treatment 4 - high Cu, medium Mo for 182 days ;
Treatment 5 - medium Cu, medium Mo for 182 days ;
Treatment 6 - low Cu medium Mo for 182 days.

The groups receiving treatments 1, 2 and 3 were slaughtered after 92 days and the remaining three treatment groups after 182 days on the treatments. During the 182 days Groups 4, 5 and 6 received approximately the same total quantity of Mo as groups 1, 2 and 3 over 92 days.

Milled veld hay and a concentrate mixture consisting of 69,4% maize meal, 22,8% commercial beef concentrate high in urea, 6,2% blood meal and 1,6% monocalcium phosphate were fed according to the procedure described in Chapter 2.

The experimental routine and analytical methods used in this trial have been given in Chapter 2. During various stages of this trial blood samples were collected for PCV, haemoglobin, plasma Cu and Mo determinations. Liver and kidney cortex samples were also collected at slaughter for mineral analyses.

The F- and t- tests were used to compare differences between treatments. Logarithmic transformations were employed to reduce differences in variance between treatments when this was indicated by Bartlett's test of homogeneity of variance (Snedecor, 1959).

RESULTS

Feed intake and composition of rations

During this trial each sheep consumed an average of 236 g of concentrate DM/day while the average veld hay intake per sheep was 770 g DM/day. The daily and total Cu, Mo and S intakes of the sheep during this trial are presented in Table 5.1. Total Mo intakes amounted to approximately the same quantity for each group, although the daily intakes per sheep differed depending on the length of the experimental period. The Cu : Mo ratios varied between 0,61 and 3,05. The average daily intakes of other nutrients per sheep were: 87 g crude protein, 10,8 g Ca, 3,3 g P, 41 mg Zn, 258 mg Mn and 707 mg Fe. The high level of Fe intake during this trial was due mainly to a high Fe content in the veld hay used 804 mg Fe/kg DM) and the contribution derived from the blood meal.

Clinical condition and mass of body and liver

No clinical sign of abnormality due to any treatment was observed

Table 5.1 Treatments and average Cu, Mo and S intakes during Trial 2

	Treatments	Duration	Copper		Molybdenum		Sulphur	
		days	per day mg	total mg	per day mg	total mg	per day g	total g
P	Pre-experimental	0	-	-	-	-	-	-
1	High Cu, High Mo	92	69,7	6412	37,7	3468	2,13	196
2	Med Cu, High Mo	92	39,2	3606	38,8	3570	2,19	201
3	Low Cu, High Mo	92	24,2	2226	39,7	3652	2,06	190
4	High Cu, Med Mo	182	67,7	12321	22,2	4040	2,17	395
5	Med Cu,Med Mo	182	40,6	7389	21,3	3877	2,22	404
6	Low Cu, Med Mo	182	25,5	4641	20,5	3731	2,05	373

during the trial. Changes in body mass at any one stage of the trial were not significantly different between treatments (Table 5.2). The mass of the fresh livers did not differ significantly between treatments and little variation in DM% of the livers was observed, as is indicated by the SE values of the means in Table 5.2.

Mineral content of body tissues

a) Liver

The amount and the concentration of Cu in the livers increased in direct proportion to the total amount of Cu consumed during the trial. This increase occurred independently of the daily amount of Cu consumed and was not influenced by the daily intake of Mo (Table 5.3). The linear increase in hepatic Cu content (Y) with increase in total Cu intake (X) is depicted in Figure 5.1. The regression $Y = 8,55 + 0,0133X$ with $n = 48$ and $r = 0,719$ describes this relationship. Average hepatic Cu retentions during the trial were therefore relatively constant and represented between 1,18 and 1,97% of the total Cu intake. Variations in liver Cu, both within and between treatments, were large, eg. in Treatment 4 the liver Cu concentrations varied between 630 and 2680 mg/kg DM (Appendix Table 1). The variations in liver Cu content within treatments tended to increase with increasing Cu intakes and this tended to reduce the statistical significance of differences between treatment means.

In contrast to Cu, the accumulation of Mo in liver was related not to the total intake of Mo but to the daily amount of Mo consumed (Table 5.4). Significantly more Mo ($P < 0,01$) was retained at the high daily intake levels than at the low, even though the total amount of Mo fed in each treatment was the same. When compared with Cu, however, the fraction of the total Mo consumed which accumulated in the liver was considerably less.

The concentration of Fe in the liver (Table 5.5) during the trial

Table 5.2 Body mass at slaughter and increase in mass during different stages of Trial 2 as well as fresh mass and dry matter percentage of livers at slaughter

Treatments	Mean body mass (kg)		Mass gain (kg)		Liver	
	92 days	182 days	1 - 92 days	92 - 182 days	fresh mass g	DM % (± SE)
1	35,0	-	6,1	-	427,8	30,3 ± 0,21
2	36,1	-	6,7	-	434,1	30,4 ± 0,24
3	36,3	-	6,9	-	432,2	30,7 ± 0,19
4	34,1	38,5	5,3	4,4	442,5	28,8 ± 0,30
5	36,2	40,7	6,3	4,5	414,3	29,0 ± 0,34
6	34,9	38,1	5,5	3,2	425,2	28.9 ± 0,27

Table 5.3 The levels and accumulation of Cu in the livers of sheep receiving different levels of Cu and Mo.

Treatments	Total Cu intake	Liver (± SE of mean) Concentration	Total Cu content	Average * Cu retention	Mean log Cu concentration	Mean log Total Cu
	mg	mg Cu/kg DM	mg	%		
P	0	346 ± 51	41 ± 6,8	0	2,500	1,563
1	6412	1013 ± 94	131 ± 13,1	1,49	2,994	2,104
2	3606	715 ± 49	94 ± 7,1	1,59	2,846	1,963
3	2226	592 ± 73	80 ± 13,3	1,97	2,730	1,846
4	12321	1730 ± 215	218 ± 26,8	1,47	3,205	2,310
5	7389	1023 ± 87	123 ± 12,8	1,18	2,998	2,076
6	4641	888 ± 82	111 ± 14,1	1,60	2,934	2,021
L S D 5%					0,162	0,176
1%					0,216	0,236

* Given as % of total Cu intakes after subtraction of pre-experimental liver Cu levels. These levels were calculated from body mass of sheep at onset of trial relative to that of pre-experimental slaughter group and its average liver Cu concentration.

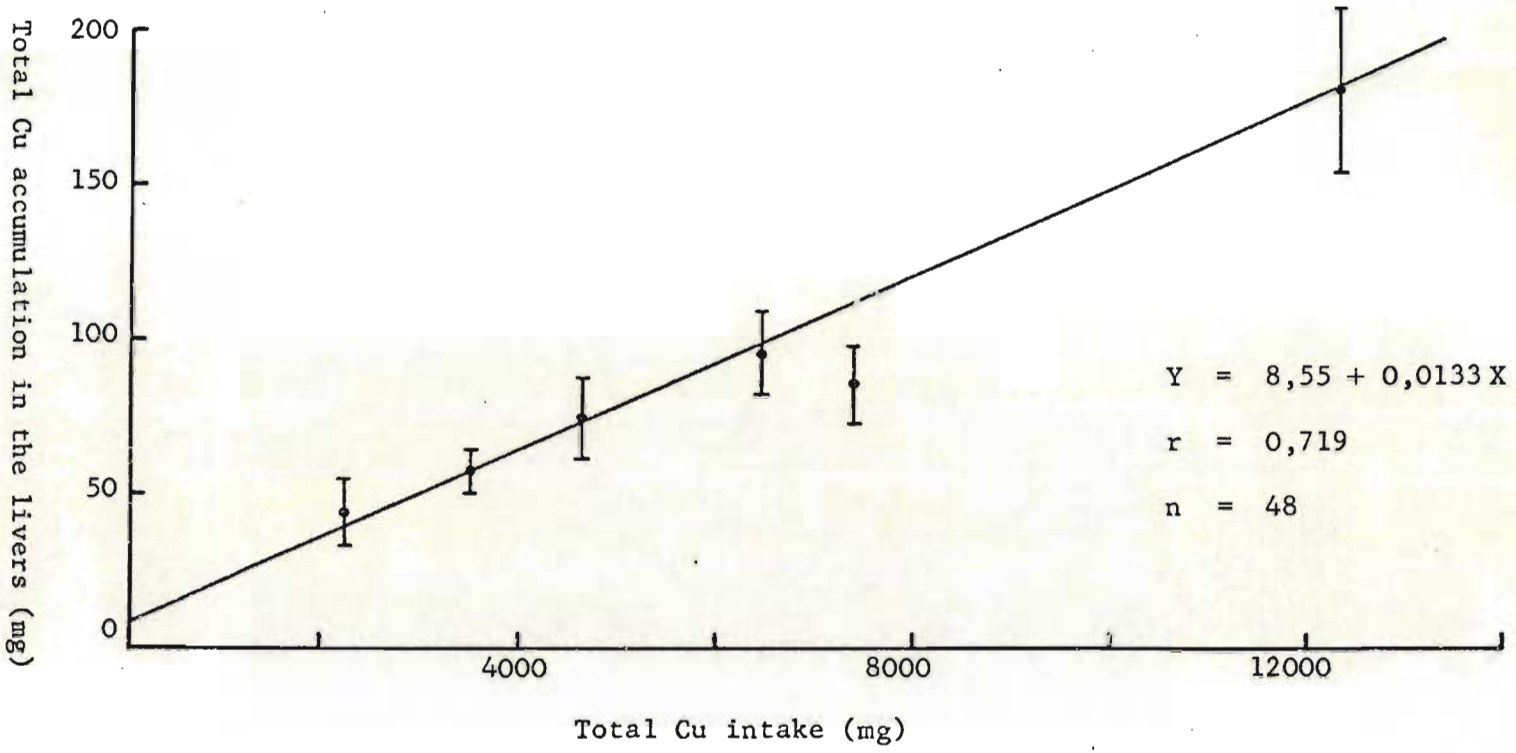

Fig. 5.1 Total Cu accumulation in the livers of sheep at different intakes of Cu. Vertical bars represent SE of means

Table 5.4 The level and accumulation of Mo in the livers of sheep receiving different dietary levels of Cu and Mo

Treatment	Intake mg	Molybdenum** (± SE of mean) Concentration mg/kg DM	Total content mg	Retention* %
P	0	$2,9^{a} \pm 0,31$	$0,34^{a} \pm 0,047$	0
1	3468	$28,1^{bd} \pm 1,60$	$3,62^{b} \pm 0,211$	0,095
2	3570	$24,4^{bd} \pm 3,01$	$3,18^{b} \pm 0,350$	0,080
3	3652	$27,6^{bd} \pm 2,41$	$3,69^{b} \pm 0,412$	0,092
4	4040	$9,5^{b} \pm 0,83$	$1,21^{a} \pm 0,123$	0,022
5	3877	$9,9^{b} \pm 0,68$	$1,19^{a} \pm 0,096$	0,022
6	3731	$9,5^{b} \pm 0,60$	$1,15^{a} \pm 0,067$	0,022

* Pre-experimental level subtracted; % of total Mo intake

** Values within columns with different superscripts denote significance at $P < 0,01$

Table 5.5 The concentration of Fe and Zn in the livers of sheep receiving different levels of dietary Cu and Mo (± SE of mean)

Treatment	Liver Fe* mg/kg DM	Liver Zn* mg/kg DM
P	$530^{a} \pm 52$	-
1	$434^{a} \pm 49$	$115^{a} \pm 4,0$
2	$552^{c} \pm 51$	$109^{c} \pm 3,3$
3	$548^{ac} \pm 45$	$104^{bf} \pm 2,2$
4	$496^{a} \pm 81$	$128^{de} \pm 6,2$
5	$400^{ad} \pm 34$	$110^{c} \pm 5,6$
6	$697^{b} \pm 45$	$110^{c} \pm 3,5$

* Different superscripts within columns designate differences between treatment averages : a - b and c - d at $P < 0,05$ and e - f at $P < 0,01$ levels of significance.

tended to increase as the Cu intakes decreased. This was particularly evident in Treatment 6 (low Cu, medium Mo) where the Fe content of the liver was significantly higher ($P<0,05$) than in the other treatments. The relatively low Fe concentration in the livers from Treatment 5 is difficult to explain; low Cu levels in the livers were also observed in this group.

The amount of Zn which accumulated in liver tended to follow the pattern of Cu storage (Table 5.5.). Significantly higher amounts of Zn were present in the livers of sheep in Treatments 1 and 4 (high Cu). The presence of Mo had no apparent effect on the final Zn content of liver.

b) Kidney cortex

The concentration of Cu in the kidney cortex increased significantly above the pre-experimental slaughter level. Differences between the other treatments were not statistically significant (Table 5.6). Although the difference in Zn level between Treatments 3 and 4 was significant ($P<0,05$), no trend according to Cu or Mo treatment was apparent.

The Mo concentration in the kidney cortex followed a similar pattern to that seen in livers, i.e. high liver concentrations in those groups receiving the high daily Mo intakes and low levels at the low daily intakes. The Fe concentrations of some kidney cortices in each of the treatments were exceptionally high, and for this reason these values are not reported here.

c) Blood plasma

The Cu and Mo levels of plasma during the trial remained more or less constant during the different stages and averages are therefore presented (Table 5.7). No statistically significant differences were observed in the plasma Cu levels between treatments. Plasma Mo levels followed the same pattern as Mo concentrations in the livers and kidney cortices, with substantially higher levels at the high daily Mo intakes than at the low intakes.

Table 5.6 Mean concentration of Cu, Mo and Zn in the kidney cortices of sheep receiving different dietary levels of Cu and Mo (± SE of mean)

Treatment	Cu* mg/kg DM	Mo* mg/kg DM	Zn* mg/kg DM
P	$11,9^{a} \pm 1,0$	-	-
1	$23,4^{c} \pm 1,3$	$59,2^{a} \pm 5,3$	$90,4 \pm 2,9$
2	$24,2^{c} \pm 1,0$	$49,1^{a} \pm 7,4$	$87,0 \pm 3,4$
3	$22,2^{c} \pm 1,0$	$64,0^{a} \pm 7,7$	$93,6^{a} \pm 4,0$
4	$21,6^{c} \pm 1,6$	$16,2^{c} \pm 3,0$	$84,3^{b} \pm 3,2$
5	$17,7^{b} \pm 0,3$	$13,1^{c} \pm 1,6$	$90,1 \pm 3,3$
6	$17,5^{b} \pm 0,5$	$12,2^{c} \pm 0,7$	$95,8 \pm 2,4$

* Different superscripts within columns designate differences between treatment averages :

a - c at $P < 0,01$ and a - b at $P < 0,05$ levels of significance.

Table 5.7 Average concentrations of Cu and Mo in plasma of sheep fed different levels of Cu and Mo

Treatments	Cu (mg/dm^3)		Mo (mg/dm^3)*	
	First 92 days	Second 90 days	First 92 days	Second 90 days
1	1,10	-	$7,5^a$	-
2	1,13	-	$7,5^a$	-
3	0,93	-	$6,4^a$	-
4	1,14	0,92	$2,5^c$	2,5
5	1,03	1,00	$3,7^c$	2,9
6	0,96	0,96	$2,8^c$	1,8

* Different superscripts within columns designate differences at $P < 0,01$ level of significance.

Haematological parameters

No changes or significant differences were observed between treatments in PCV and haemoglobin levels (Table 5.8). The PCV levels were relatively low though faecal worm egg counts revealed no traces of internal parasite infestation as a possible explanation for this.

DISCUSSION

The presence of S is considered to be essential to the Cu - Mo interaction (Dick, 1954; Dick et al., 1975) and S is also believed to reduce Mo retention in the body. A dietary S intake of less than 2 g/ sheep/day was effective in reducing hepatic Cu retention, presumably by its effect on the Cu - Mo interaction (Dick, 1954; Ross, 1966; Marcilese et al., 1970; Bremner & Young, 1978). However, Grace & Suttle (1979) concluded that diets low in degradable SO_4^{2-} content did not favour the formation of thiomolybdates, compounds suggested by Suttle (1974b) and Dick et al. (1975) to bind Cu. Dick (1954) had previously found that increasing levels of SO_4^{2-} (rising from 1 g to 6 g/sheep/day) served to decrease hepatic Cu retention. The extent of the reduction depended on the concurrent intake of Mo. When the Cu and the SO_4^{2-} intakes were held constant, increased amounts of dietary Mo (from 20 mg to 100 mg/sheep/day) had little effect on liver Cu retention by sheep.

In the present trial the daily S intakes of all sheep were held constant at about 2,0 g per day, a level shown to be adequate for the promotion of the Cu - Mo interaction. The Cu and Mo intakes were varied in the different treatments giving Cu : Mo ratios over the range 0,6 to 3,0. Under these circumstances Mo failed to affect hepatic Cu retention. This finding is contrary to the results obtained in Trial 1, and unexpected in the light of reports in the literature. This lack of an effect of Mo may possibly be due to the inadequate formation of thiomolybdates, and consequent low binding of dietary Cu.

Table 5.8 Packed cell volume (PCV) and haemoglobin (Hb) values of blood from sheep fed different levels of Cu and Mo

Treatments		Date of collection 27/7	7/9	20/10	7/12
1	PCV	25,0	22,5	-	-
	Hb	10,6	10,2	-	-
2	PCV	23,5	21,9	-	-
	Hb	9,8	9,5	-	-
3	PCV	24,1	24,9	-	-
	Hb	10,5	10,3	-	-
4	PCV	22,3	20,8	25,6	24,8
	Hb	9,6	9,2	10,3	9,9
5	PCV	24,9	21,1	24,6	26,1
	Hb	10,4	10,4	9,8	11,1
6	PCV	20,6	24,8	25,3	24,8
	Hb	9,5	10,1	9,9	10,3

The linear increase in Cu accumulation in the liver with the increase in Cu intake observed in this trial corresponded well with similar observations by Dick (1954), Hemingway & MacPherson (1967) and with the two treatments without Mo in Trial 1. However, much higher dietary Cu levels were required in the present trial to achieve liver Cu levels approximating those reported by Dick (1954) and Hemingway & MacPherson (1967). The proportion of dietary Cu retained in the liver in Trial 2 was between 1,2 and 2,0% as compared to 3,3 to 5%, 2,96 to 3,01%, 4,4% and 3,2 to 8,3% reported by Dick (1954), MacPherson & Hemingway (1965), Hemingway & MacPherson (1967) and Suttle et al. (1978) respectively. With the use of the hypocupraemic ewes and the Cu depletion-repletion technique, Suttle (1974a) found that the true availability of dietary Cu in the body of the sheep varied between 4,1 and 11,4%

The relatively low retention of dietary Cu in the livers of the sheep used in the present trial is difficult to explain. With the fixed level of dietary S, the two levels of Mo tested had no apparent effect on Cu storage in the liver. However, since higher amounts of dietary Cu were necessary to achieve Cu levels in the liver similar to those reported by Dick (1954), it is possible that the Mo : SO_4^{2-} combination did have some inhibiting effect on the body storage of Cu. On the other hand, the reduced liver storage may have reflected other influences, such as genetic factors influencing Cu metabolism in the body. This aspect will be considered presently.

A dietary S level of just above 2 g/sheep/day is sufficient to meet the S requirements of a sheep (Pope, 1975). Hume & Bird (1970) found 1,95 g S/day to be adequate for supporting maximum protein production in the rumen while Huisingh & Matrone (1976) accepted 0,28% S in the diet as the requirement for growing lambs fed a purified diet containing urea. A dietary S level of above 2 g/kg feed was suggested by Suttle (1974c) as essential for eliciting a depressing effect of S on Cu absorption. Huisingh & Matrone (1976) suggested the formation of CuS to be more significant in rendering Cu unavailable to the ruminant than the interaction of Cu with Mo. Availability of

Cu will depend on the pool of ruminal S^{2-}. Huisingh & Matrone (1976) pointed out that Mo affected the pool of sulphide by inhibiting the reduction of SO_4^{2-} to S^{2-}. However, with methionine as the source of S, Mo did not inhibit S^{2-} formation. They observed that Mo aggravated a state of Cu deficiency in sheep when methionine was the source of S but alleviated the Cu deficiency when SO_4^{2-} was supplying the S. In the present trial the S was probably mainly in the amino acid form which could lead to considerable S^{2-} formation even at a level of about 2 g/sheep/day. This may have contributed to the low Cu retention observed, similar to the observations by Huisingh & Matrone (1976).

It was not possible to establish to what extent the high Fe intakes during the trial contributed to the low liver Cu retention. Standish et al. (1968) reported that high levels of dietary Fe (at 400 mg Fe/kg DM and above) decreased the availability of dietary Cu to cattle. Abdellatif (1968) made similar observations in sheep but tested the effect only at very high Fe intakes. The decrease in liver Fe concentrations with increased Cu intakes, observed in the present trial, indicated that some interaction must have taken place between Cu and Fe.

The daily Zn intake of the sheep (41 mg/sheep/day) was well below the levels of 220 and 420 mg Zn/kg diet at which Bremner et al. (1976) observed decreased hepatic Cu retentions due to the Zn intakes. A tendency for hepatic Zn concentrations to increase with increased Cu intakes was observed in the present trial. This is in agreement with observations by Suttle & Mills (1966) and Gibb, Pond, Kallfelz, Tasker, Van Campen, Krook & Visek (1974) on pigs, and suggests the existence of some interaction between Cu and Zn in the body. The increase in liver Zn concentrations at high Cu intakes as observed by Suttle & Mills (1966) was considered to be a manifestation of Cu-induced Zn deficiency in pigs (Bremner & Marshall, 1974).

British sheep breeds have been found to be more susceptible to Cu toxicosis than Corriedales, with the Merino being the least susceptible

of the sheep breeds tested (Edgar et al., 1941; Marston & Lee, 1948). To what extent breed differences contributed to the differences in rate of Cu retentions is open to speculation. Corriedales were used in the present trial and SA Mutton Merinos in Trial 1. Dick (1954) used crossbreds in his trials because of their higher susceptibility to Cu toxicity than Merinos, while MacPherson and Suttle used Scottish Black-faced sheep. Wiener & Field (1969) reported variations depending on breed, in the relationship between liver Cu levels and hypocuprosis observed in British sheep breeds. A higher caeruloplasmin turnover rate in cattle than in sheep was suggested to be a possible reason for the higher sensitivity of cattle to Mo toxicity than sheep (Marcilese, Figueiras & Valsecchi, 1976) and for the higher sensitivity of sheep to Cu toxicity than cattle (Ward, 1978). Genetic differences in caeruloplasmin turnover rates between sheep breeds may therefore also be a reason for differences in resistance to Cu toxicity between sheep breeds.

Variations in liver Cu content at the onset of the trial and possibly uneven Cu intakes, because of the group feeding regime, must have contributed to the wide variation observed in hepatic Cu content within treatments. This may explain the lower Cu and Fe retentions observed in Treatment 5 of this trial as compared with the other treatments. However, differences within treatment groups in the genetic ability of the sheep to absorb and/or to retain Cu may have been responsible for some of the variation. Todd et al. (1962) observed a wide variation in hepatic Cu concentrations in sheep receiving the same dietary Cu levels. This would explain why usually only a small proportion of a flock succumb to Cu toxicity (Todd, 1969). MacPherson & Hemingway (1965) mentioned a tendency in their trials for there to be a lower liver Cu storage in those sheep which survive for longer periods on diets high in Cu. It therefore seems possible that genetic differences due to sheep breeds may have been partly responsible for the lower hepatic Cu retentions observed in the present trial as compared with those of other workers.

Corbett, Saylor, Long & Leach (1978) suggested that the close relationship between dietary Cu and liver Cu as observed by Dick (1954) indicated the existence of very little homeostatic control

over Cu absorption by sheep; this is contrary to the position reported for other species (Beck, 1963; Milne & Weswig, 1968; Fisher et al., 1972; Hedges & Kornegay, 1973). However, Suttle et al. (1978) noticed some evidence for the existence of homeostatic control in the Cu absorption and retention in sheep at high Cu intakes. In the present trial this linear relationship extended to well within the range of hepatic Cu concentrations at which Cu toxicity can be expected (Harker, 1976) and well above the levels recorded by Suttle et al. (1978). Dick (1954) had also observed a slightly decreased Cu retention at his highest level of dietary Cu intakes. Neethling, Brown & De Wet (1968) measured not only a reduced absorption of Cu^{64} at high doses, which they ascribed to some regulatory mechanism at high Cu intakes, but also increased Cu absorptions in Cu-depleted sheep. In view of these observations by Neethling et al. (1968), true Cu availability estimates calculated with the use of the Cu depletion-repletion technique (Suttle, 1974a) may not give a true reflection of Cu availability under natural conditions.

Mo concentrations in the liver, kidney cortex and plasma in the present trial seemed to follow the level of Mo intake. This implies that the Mo concentration in these organs may be used as parameters of the Mo status of sheep. Cunningham & Hogan (1959) found the Mo concentrations of the bone, kidneys and spleen to be related to Mo intakes. Lesperance & Bohman (1963) suggested the use of Mo levels in plasma and liver as indicators of the Mo status and the danger of Mo toxicity in cattle. Symptoms of molybdenosis per se, viz. diarrhoea, anorexia, etc., as observed in cattle, are seldom encountered in sheep (Cunningham & Hogan, 1959; Ward, 1978). Furthermore, the observation in the present study that the Mo levels in the organs and plasma tended to vary depending on levels of daily Mo intake, rather than total Mo intake during the trial, indicates that Mo in the sheep body is fairly transient, irrespective of Cu intake. This would limit the value of tissue Mo concentrations in predicting the overall Mo status of an animal, though high plasma Mo levels would indicate excessive Mo intakes on a short term basis (Lesperance & Bohman, 1963).

Molybdenosis as observed in sheep is usually expressed in the form of induced hypocuprosis. The presence of S in the diet was found to be essential to elicit this action of Mo on Cu in the body (Dick, 1954; Suttle, 1974c). However, the addition of S to a diet is also accompanied by a decrease in tissue Mo concentrations (Dick, 1956). Plasma and tissue Mo levels would, therefore, be of little value in predicting induced hypocuprosis in sheep. In fact, low plasma and tissue Mo levels may even be potentially more dangerous than high levels. The use of a Cu : Mo ratio in feed as suggested by Miltimore & Mason (1971) for predicting the risk of molybdenosis in cattle, would also be unreliable for predicting induced hypocuprosis in sheep if the S content of the diet is unknown. This is well demonstrated in the present trial where six different ratios of Cu : Mo were fed, without affecting hepatic Cu retentions.

The low PCV percentages associated with approximately normal levels of haemoglobin observed in this trial may indicate a state of anaemia which may reflect a Cu deficiency due to high Mo intakes. This is unlikely though, considering the levels of Cu in the blood and in the liver and the high Cu intake. Dietary Fe intakes were also very high and lack of Fe is probably not responsible for the low PCV values. It is unlikely, therefore, that treatments used during this trial were responsible for the state of anaemia, indicated by the low PCV recorded.

CHAPTER 6

TRIAL 3 : INFLUENCE OF DIETARY S ON CU AND MO METABOLISM IN SHEEP RECEIVING HIGH LEVELS OF CU AND MO

INTRODUCTION

From the literature it appears to be the accepted practice to supply additional S whenever Mo is used in the treatment or prevention of Cu toxicosis in sheep. Garner (1963), for instance, recommended the daily addition of between 0,3 and 1 g sodium thiosulphate with 50 to 500 mg ammonium molybdate to control Cu toxicity in sheep. The importance of the S content in the basic diet was demonstrated in Trial 1. The failure to reduce liver Cu concentrations with high levels of Mo supplementation in Trial 2 was attributed, at least partially, to the low S content of the diets. Suttle (1975b) pointed out that natural variations in S and Mo content of grazing could influence the Cu status of the grazing animal, while Ward (1978) suggested the use of the protein content of a ration as an indication of the S content of the diet.

A trial was carried out in which different levels of S were supplied after a period of Cu accumulation in the body, to determine the effect of level of S intake on the Cu and Mo metabolism of sheep receiving high levels of these minerals.

PROCEDURE

Experimental animals, treatments and procedure

Thirty S.A. Mutton Merinos, age 1½ to 2 years, were divided into five groups consisting of five rams and one wether per group. All groups received a diet high in Cu and Mo but low in S for the first 42 days of the trial (Figure 6.1). One group was slaughtered after

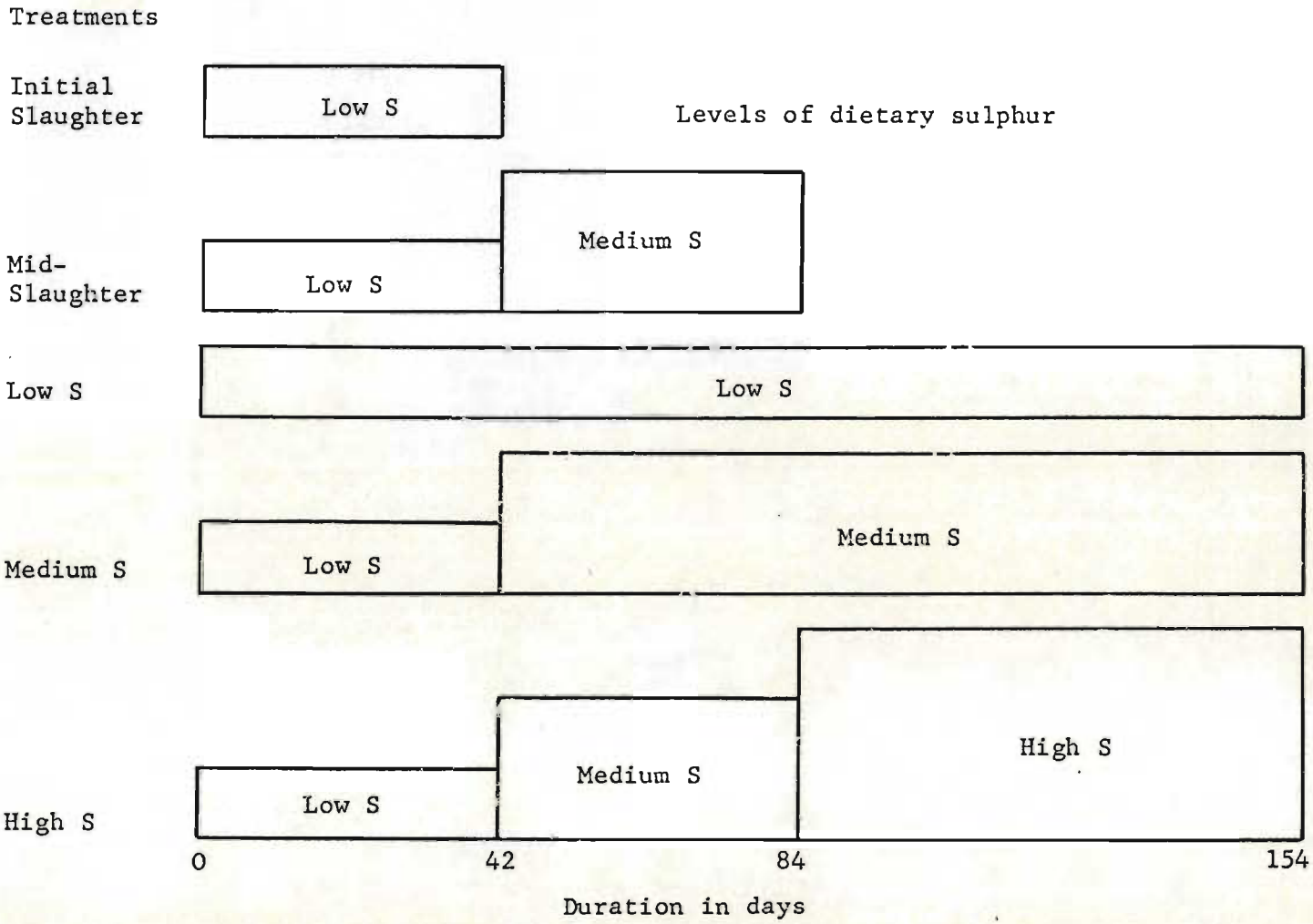

Fig. 6.1 Diagrammatic exposition of duration and different levels of dietary S during Trial 3.

42 days (Initial slaughter), one group (Low S) was kept on the low S ration to the end of the trial while the remaining three groups received the medium level of additional S for a further 42 days. At this stage one of the latter groups was slaughtered (Mid-slaughter), one group was left on the medium S ration (Medium S) while the last group received an even higher level of additional S (High S). These three remaining groups were slaughtered after a further 70 days on their respective treatments. The treatments applied and the actual Cu, Mo and S intakes are given in Table 6.1.

Veld hay and a concentrate mixture consisting of 86% maize meal and 4,6% each of urea, salt and monocalcium phosphate were fed to the sheep as described in Chapter 2.

The experimental routine was similar to that described in Chapter 2. GOT concentrations were determined towards the end of the trial to monitor tissue damage in the body (Todd, 1969). Livers, kidneys, spleens, lungs, testes and muscle samples were collected at slaughter and prepared for further analyses.

The chemical and statistical methods as described in Chapter 2 were used in this trial.

RESULTS

Feed intake and composition of rations

An average intake per sheep of 893 g DM in the form of veld hay and 176 g of the concentrate mixture on a dry basis, was recorded. Treatments and average daily Cu, Mo and S intakes for the various stages of the trial are presented in Table 6.1. The average ratio of Cu : Mo in the ration was 1,63 : 1. The average daily intake per sheep of other nutrients during the trial were : 96 g crude protein 9,5 g Ca, 2,9 g P, 29 mg Zn, 256 mg Mn and 493 mg Fe. The S content of the hay in this trial was higher than expected, resulting in daily S intakes higher than those in Trial 2, although it had been planned

Table 6.1 Treatments, average Cu and Mo intake/sheep/day during the experiment and average S intakes/sheep/day during the different stages of Trial 3

Treatments	Duration	Copper		Molybdenum		Sulphur					
						First 42 days		Second 42 days		Last 70 days	
	days	per day mg	Total mg	per day mg	Total mg	per day g	Total g	per day g	Total g	per day g	Total g
Initial slaughter	42	53,1	2230	33,9	1424	2,88	121	-	-	-	-
Mid-slaughter	84	55,9	4696	34,7	2915	2,88	121	3,86	162	-	-
Low S	154	58,2	8963	35,1	5405	2,88	121	2,98	125	2,90	203
Medium S	154	56,3	8670	33,1	5097	2,88	121	3,86	162	4,01	281
High S	154	53,6	8254	32,9	5067	2,88	121	3,86	162	5,30	371

to duplicate the S intakes of Trial 2. Due to some difficulty with the feed mixer, Mo levels of the concentrate mixture during the last 28 days of the trial were slightly lower than during the preceding period.

Clinical condition and mass of body and organs

No clinical signs of abnormality due to any treatment were observed. One sheep from the Mid-slaughter group was lost due to theft. A drop in body mass was observed during the pre-experimental 42 days, but the mass of all groups increased slightly for the rest of the experimental period (Table 6.2). No statistically significant differences in body and organ mass or organ DM percentage was observed between treatments (Table 6.3). Slight but inconsistent differences occurred in testes mass and DM percentage. The DM percentages of the organs showed very little variation as can be seen from the SE of the means.

Mineral content of body tissues

a) Liver

Very little accumulation of Cu or Mo took place in the liver after the first 42 days of the trial (Table 6.4). The addition of S to the diet resulted in negative Cu retentions in two treatment groups, the Mid-slaughter and High S groups. These Cu levels were significantly ($P<0,01$) lower than those found in the low S group. Mo concentrations in the liver decreased during the period when dietary S intakes were increased, below the level of the Initial slaughter group. Negative liver Mo retentions were observed in all groups as compared to the Initial slaughter group, though differences between groups were insignificant.

b) Kidney cortex

Significant increases in cortex Cu concentration were observed when

Table 6.2 The average body mass of the sheep and changes in mass between the various stages of Trial 3

Treatment	Initial mass	Mass at 42 days		Mass at 84 days		Mass at 154 days	
		Mass	Gain*	Mass	Gain*	Mass	Gain*
	kg	kg	kg	kg	kg	kg	kg
Initial slaughter	49,3	44,5	-4,8	-	-	-	-
Mid - slaughter	52,4	46,5	-5,9	47,0	0,5	-	-
Low S	52,4	47,8	-4,6	49,3	1,5	50,7	1,4
Medium S	51,8	47,2	-4,6	49,3	2,1	51,1	1,8
High S	52,0	47,2	-4,8	49,5	2,3	50,8	1,3

* Difference from previous period.

Table 6.3 The average mass of the different organs (fresh) and the dry matter percentages of the organs at slaughter

Organs	Initial slaughter	Mid-slaughter	Treatments Low S	Medium S	High S
Liver					
Fresh (g)	520	509	477	503	526
Dry matter (%) **	27,5 ± 0,47	28,3 ± 0,23	28,3 ± 0,40	28,2 ± 0,34	28,3 ± 0,35
Kidneys *					
Fresh (g)	111	115	110	111	115
Spleen					
Fresh (g)	124	99	101	99	107
Dry matter (%)**	21,7 ± 0,19	22,7 ± 0,24	21,6 ± 0,18	21,9 ± 0,35	21,1 ± 0,14
Lungs					
Fresh (g)	459	490	486	485	523
Dry matter (%) **	19,7 ± 0,31	20,5 ± 0,20	19,9 ± 0,36	20,6 ± 0,45	20,8 ± 0,44
Testes					
Fresh (g)	256	153	203	255	220
Dry matter (%) **	14,6 ± 0,20	16,1 ± 0,49	13,8 ± 0,05	13,2 ± 0,21	13,8 ± 0,28

* Kidney cortices used in analyses

** ± SE of means

Table 6.4 The levels and accumulation of Cu and Mo in the livers of sheep receiving different levels of S (± SE of means)

Treatment	Copper*** Intake	Liver		Retention*	Molybdenum *** Intake	Liver		Retention*
		mg/kg DM	mg	%	mg	mg/kg DM	mg	%
Initial slaughter	0**	379 ± 59	53,7	0	0**	$10,7^{a}$ ± 1,39	1,49	0
Mid - slaughter	2466	317^{c} ± 52	49,6	-0,17	1491	$6,4^{b}$ ± ,52	0,98	-0,034
Low S	6733	515^{a} ± 26	69,7	0,24	3981	$8,6^{a}$ ± 0,76	1,15	-0,009
Medium S	6440	458 ± 43	64,1	0,16	3673	7,6 ± 0,81	1,06	-0,012
High S	6024	342^{c} ± 36	51,3	-0,04	3643	7,7 ± 0,83	1,14	-0,010

* Pre-experimental level subtracted ; % of total Cu intake

** Cu and Mo intakes during first 42 days deducted

*** Values within columns per trial with different superscripts denote significance :
a - b at $P < 0,05$ a - c at $P < 0,01$ levels of significance

additional dietary S was supplied above the Low S treatment (Table 6.5). The Cu concentrations in the cortices of the Mid-slaughter and Medium S groups (which received the same level of S supplementation/day) were similar, even though the treatment period for the latter was 70 days longer than for the Mid-slaughter group. A combined regression equation with a correlation of r = 0,916 was observed between the Cu and Mo concentrations in the kidney cortices of the three treatments receiving additional S above that of the Low S level, viz. the Mid-slaughter, Medium S and High S treatments (Figure 6.2). An average atomic ratio of 2,15 was observed between the Cu and Mo concentrations in these groups. For the Initial slaughter and Low S treatment groups, the average correlation between Cu and Mo was r = -0,02.

c) Spleen, lungs, muscle and testes

The effect of dietary S supplementation during the trial on Cu and Mo concentrations in the spleen, lungs, muscle and testes is given in Table 6.6 and Figure 6.3. Statistically significant differences ($P < 0,05$) in Cu concentration were observed in the spleen between the Initial slaughter and Medium S groups and in the testes ($P < 0,01$) between the earlier slaughter groups and those kept in the trial for 154 days. The feeding of additional S was associated with significant reductions in the Mo concentrations in all the tissues. The effect of additional S on Mo concentrations in these tissues showed a non-linear pattern with a marked reduction in Mo concentration between the Low S and Medium S treatments but without any additional reduction due to further S supplementation.

No statistically significant differences in the Zn and Fe concentrations of the livers, kidneys, spleens, lungs, muscles or testicles were observed which could be related to any treatment effect.

d) Plasma

Average plasma Cu and Mo concentrations for the different stages of

Table 6.5 The influence of different levels of dietary S on the concentration of Cu and Mo and the Cu : Mo ratio in the kidney cortices (± SE of means)

Treatment	Kidney Cortex		
	Cu mg/kg DM	Mo mg/kg DM	Cu : Mo** ratio
Initial slaughter	$16,0^{b} \pm 0,89$	$13,9 \pm 2,24$	$1,15 \pm 0,33$
Mid-slaughter	$28,3^{a} \pm 2,94$	$14,9 \pm 2,41$	$1,90 \pm 0,12$
Low S	$18,0^{be} \pm 0,93$	$17,1 \pm 2,27$	$1,05 \pm 0,18$
Medium S	$26,6^{ac} \pm 2,50$	$11,8^{c} \pm 1,67$	$2,25 \pm 0,10$
High S	$40,3^{ad} \pm 4,70$	$18,8^{d} \pm 1,83$	$2,14 \pm 0,10$

* Different superscripts within columns designate differences between treatment averages : a - b at $P < 0,01$ and c - d and c - e at $P < 0,05$ levels of significance

** Atomic ratio

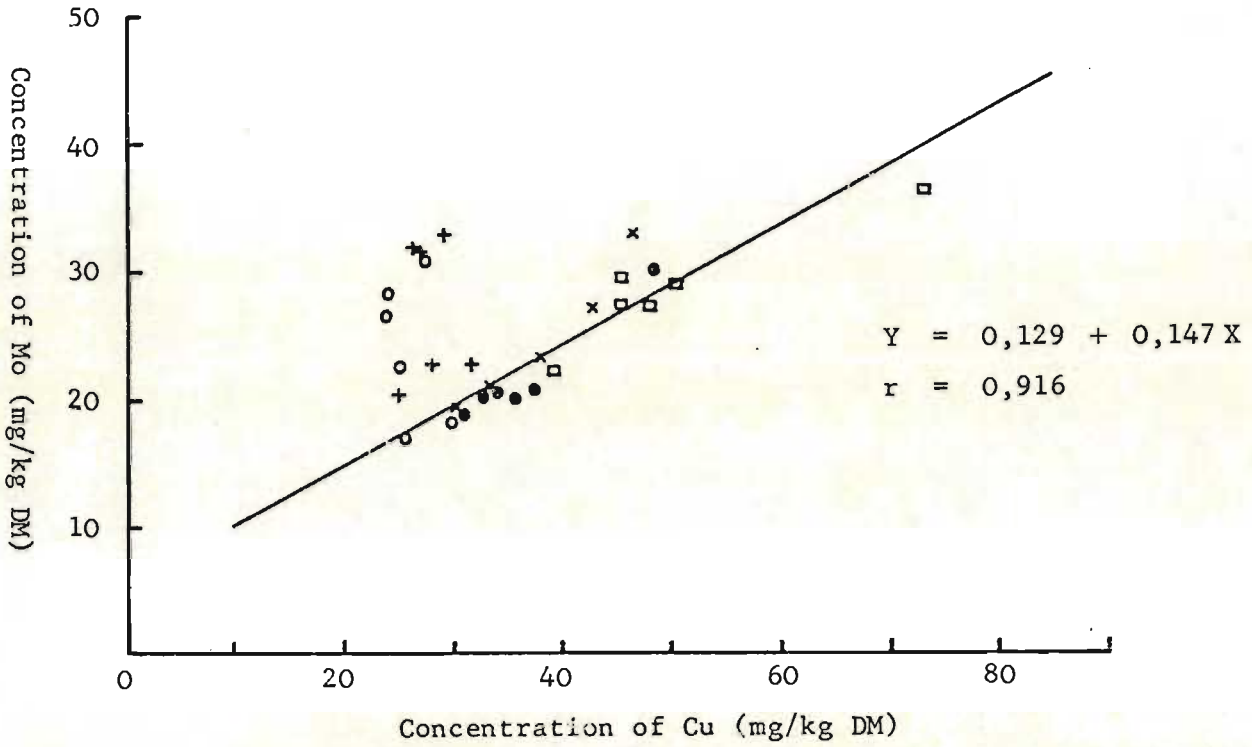

Fig. 6.2 The relationship between Cu and Mo in the kidney cortices of sheep. A combined regression equation for the groups receiving additional S, viz. ▫ high S, ● med S and x mid-slaughter. The correlation between the groups without addition S, o pre-slaughter and + low S was r = -0,02.

Table 6.6 The effect of S supplementation on the Cu and Mo levels (DM basis) in organs and tissue (± SE of means)

Treatment	Copper *				Molybdenum *			
	Spleen mg/kg	Lungs mg/kg	Muscle mg/kg	Testes mg/kg	Spleen mg/kg	Lungs mg/kg	Muscle mg/kg	Testes mg/kg
Initial slaughter	$6,4^{a} \pm 0,17$	$13,5 \pm 0,64$	$3,22 \pm 0,49$	$6,2^{a} \pm 0,42$	$7,1^{a} \pm 0,50$	$7,4^{2} \pm 0,94$	$2,07^{a} \pm 0,47$	$5,1^{a} \pm 0,90$
Mid-slaughter	$5,3 \pm 0,26$	$14,5 \pm 0,52$	$3,18 \pm 0,35$	$6,8^{a} \pm 0,36$	$1,7^{c} \pm 0,65$	$1,6^{c} \pm 0,07$	$0,52^{b} \pm 0,10$	$1,3^{c} \pm 0,09$
Low S	$4,4 \pm 0,25$	$10,3 \pm 0,43$	$3,35 \pm 0,17$	$8,7^{c} \pm 0,25$	$5,1^{a} \pm 0,79$	$6,2^{a} \pm 0,72$	$1,10 \pm 0,24$	$3,5^{a} \pm 0,51$
Medium S	$4,2^{b} \pm 0,13$	$9,0 \pm 0,55$	$3,08 \pm 0,11$	$9,2^{c} \pm 0,77$	$1,3^{c} \pm 0,14$	$1,5^{c} \pm 0,19$	$0,50^{b} \pm 0,14$	$1,4^{c} \pm 0,11$
High S	$5,6 \pm 0,38$	$8,3 \pm 0,63$	$3,73 \pm 0,18$	$9,0^{c} \pm 0,19$	$1,4^{c} \pm 0,10$	$1,7^{c} \pm 0,40$	$0,42^{c} \pm 0,13$	$1,5^{c} \pm 0,47$

* Different superscripts within columns designate differences between treatment averages :
a - b at $P < 0,05$; a - c at $P < 0,01$ levels of significance.

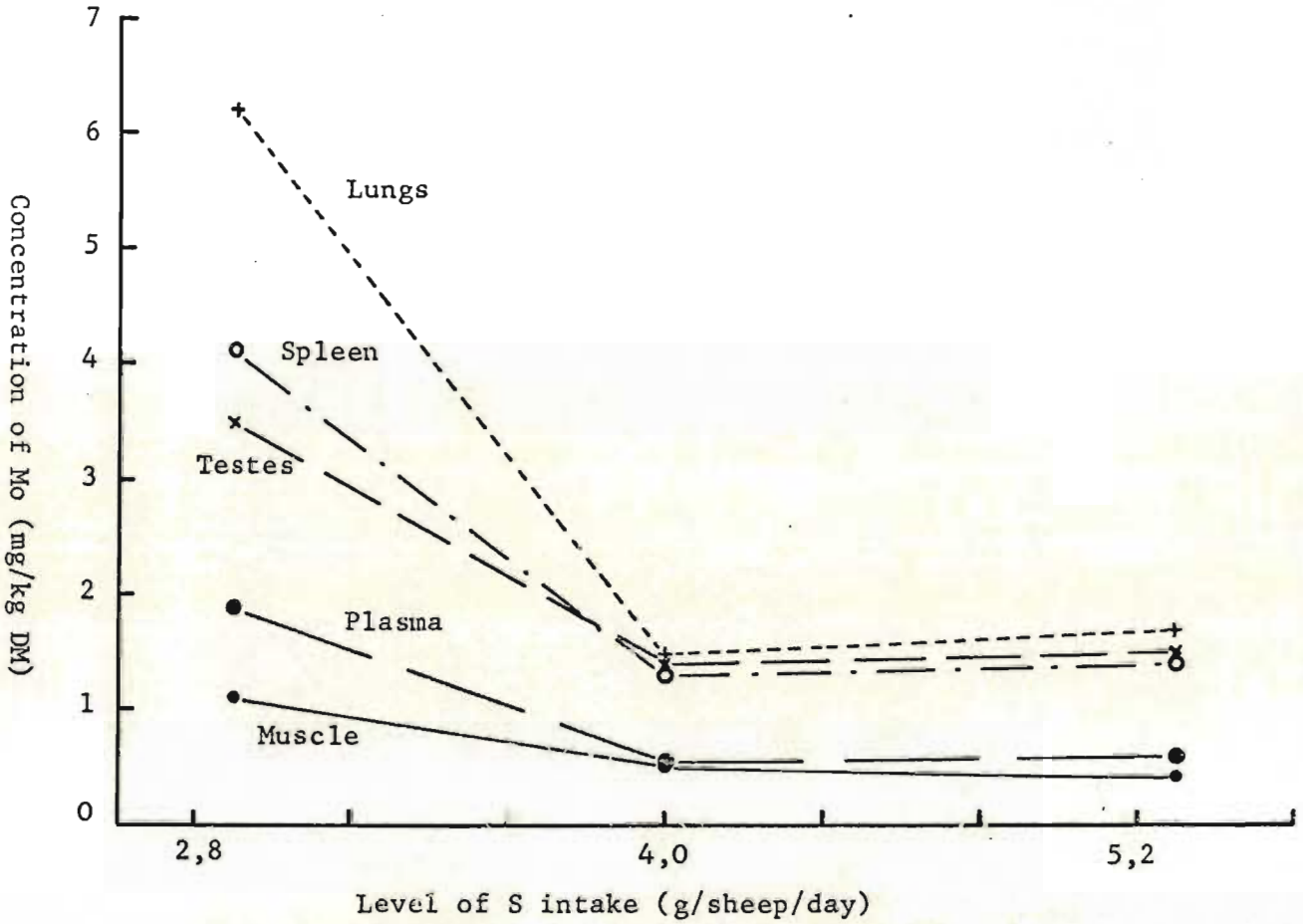

Fig. 6.3 Influence of level of S intake on the Mo concentrations of the spleen, lungs, muscle, testes and plasma of sheep

the trial are presented in Table 6.7. Changes in plasma Mo and Cu concentrations are depicted in Figures 6.3 and 6.4 respectively. The addition of S, i.e. during the second and third stages, resulted in increased plasma Cu and decreased plasma Mo concentrations. The difference in plasma Cu levels between the control and the other groups receiving additional S, was not statistically significant during the second 42 day period, but was so during the last 70 days of the Trial.

Haematological parameters and serum enzymes

No changes or significant differences were observed between treatments in the haemoglobin and PCV values, during the various stages of the trial (Table 6.8). Haemoglobin levels varied between 11 and 12,7 g/100 cm^3 blood and PCV between 27,2 and 31,6%. The GOT levels in serum were determined towards the end of the trial (Table 6.9). These values varied between 42 and 56 IU/dm^3 with no significant differences between treatments. Only one sheep, in the High S group, had a GOT value of 104 IU/dm^3 shortly before it was slaughtered.

DISCUSSION

A relatively low rate of hepatic Cu retention (0,24%) was observed at an S intake of 2,9g/sheep/day. Above this S level negative hepatic Cu retentions were obtained. Reduced liver Cu levels have been recorded at S and Mo levels similar to those used in the Low S treatment of this trial (Dick, 1954 ; Harker, 1976). At the levels of Cu, Mo and S used in Trial 3, it may be concluded that the low hepatic Cu retentions were to a great extent due to the Cu - Mo - S interaction.

It is evident from the results that liver Cu retentions can be restricted, or can even be reduced to negative values at a dietary Cu : Mo ratio of approximately 1,63. These results substantiate the suggestion by Miltimore & Mason (1971) that the danger of Cu deficiencies due to dietary Mo existed at Cu : Mo ratios of less than 2,0.

Table 6.7 The effect of S supplementation on the plasma Cu and Mo concentrations during various stages of the trial.

Treatments	Plasma Cu (mg/dm^3) *			Plasma Mo (mg/dm^3) *		
	First 42 days	Second 42 days	Last 70 days	First 42 days	Second 42 days	Last 70 days
Initial slaughter	1,28	-	-	2,43	-	-
Mid-slaughter	1,25	1,39	-	2,39	$0,54^{c}$	-
Low S	1,34	1,25	$0,96^{a}$	2,26	$1,88^{a}$	$1,92^{a}$
Medium S	1,36	1,40	$1,22^{c}$	2,39	$0,53^{c}$	$0,48^{c}$
High S	1,29	1,36	$1,18^{c}$	2,34	$0,60^{c}$	$0,49^{c}$

* Different superscripts a - b within columns designate differences at $P < 0,01$ level of significance

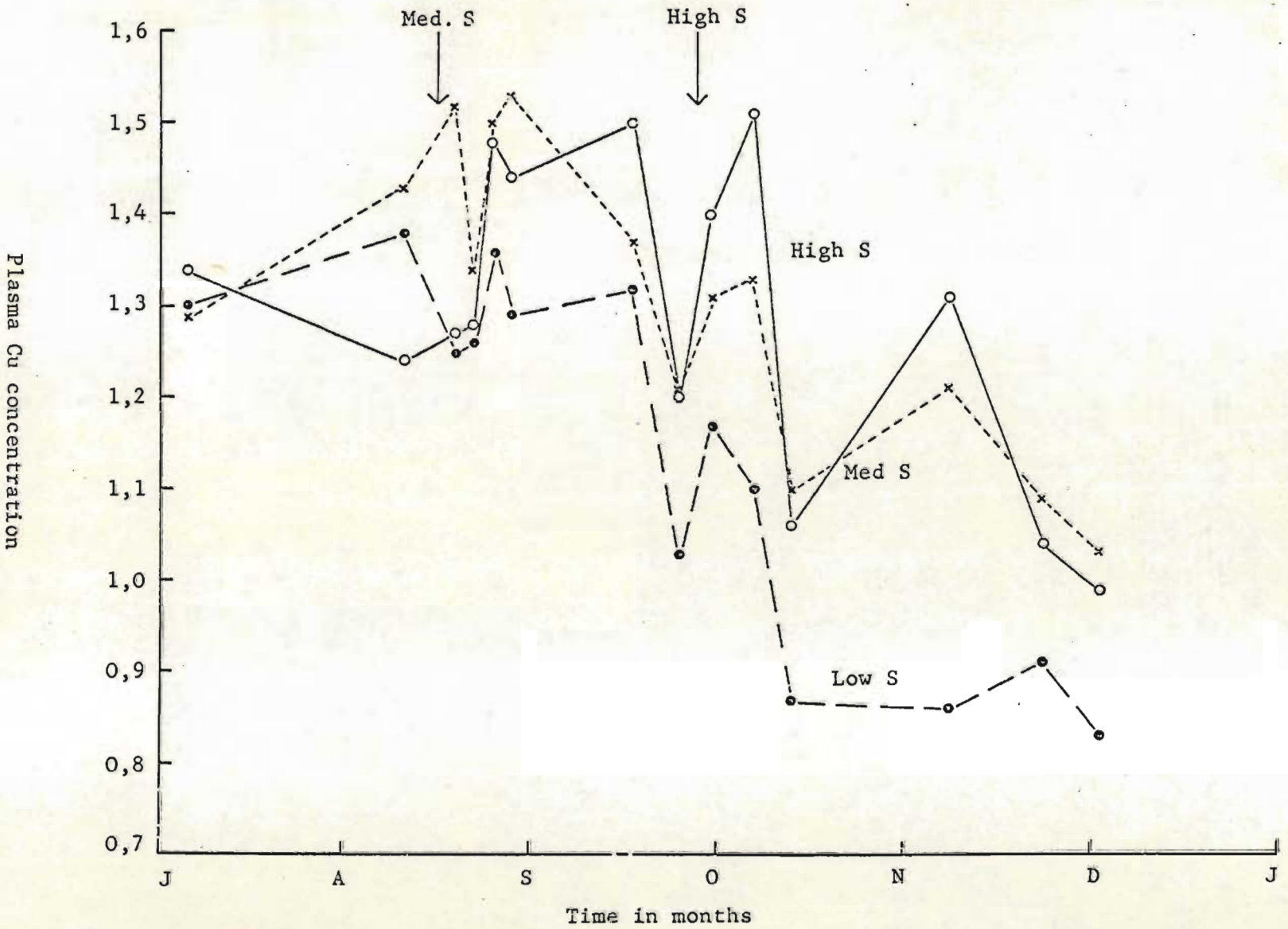

Fig. 6.4 Influence of level of S intake on the Cu concentration in plasma at different stages of Trial 3

Table 6.8 The influence of various levels of dietary S on the packed cell volume (PCV) and haemoglobin (Hb) levels in blood during Trial 3

Treatment	Data of collection 5/7 PCV %	5/7 Hb g/100cm^3	10/8 PCV %	10/8 Hb g/100cm^3	16/9 PCV %	16/9 Hb g/100cm^3	2/12 PCV %	2/12 Hb g/100cm^3
Initial slaughter	30,6	12,0	27,5	10,6	-	-	-	-
Mid - slaughter	29,5	11,8	26,4	10,5	29,4	11,5	-	-
Low S	28,9	11,6	27,2	10,4	29,2	11,2	28,7	11,0
Medium S	28,7	11,6	28,7	11,3	27,8	11,0	29,7	11,3
High S	31,6	12,7	28,8	11,4	31,3	12,0	30,3	11,5

Table 6.9 The average serum GOT levels of sheep during the last two months of Trial 3 (± SE of means)

Treatments	Serum G O T levels (IU/dm^3) Low S	Medium S	High S
Dates			
16/11	51,7 ± 4,0	46,5 ± 2,5	49,7 ± 1,4
6/12	48,3 ± 5,8	42,7 ± 3,6	55,8 ± 9,6*

* The concentration of serum G O T in one sheep was 104 IU/dm^3

However, it is clear that the level of S intake has an important influence on the degree of response to be expected, as has also been concluded in Trial 2, and cannot be ignored. Differences in S levels used, may explain why different ratios of Cu : Mo, viz. 2 : 1 (Miltimore & Mason, 1971), 5 : 1 (Alloway, 1973 ; Pope, 1975) 7 : 1 (Case, 1974) had been suggested as safe limits against molybdenosis. Suttle (1974b) suggested the formula "product log (Mo concentration) x log (S concentration)" for predicting the effects of dietary Mo and S on the availability of Cu in feeds, thus taking into account the S content of the food.

Although the importance of a knowledge of dietary S levels seems obvious, different factors can influence the amount and availability of S in the body. Suttle (1975b) mentioned the possibility that some S may escape ruminal degradation and not participate in the interactions with Mo. Hume & Bird (1970) reported substantial endogenous additions of S through the saliva to the S pool in the rumen. Scaife (1956) suggested that protein catabolism in the body may contribute to the S pool involved in the Cu - Mo - S interaction. Although Marcilese et al. (1969) proved that intravenously injected SO_4^{2-} did not contribute to the Cu - Mo interaction in the body, it seems possible that S from protein catabolism could recycle back into the rumen where it can influence the Cu - Mo interaction. The form in which S is ingested by the animal, eg. as amino acid S or SO_4^{2-} (Huisingh & Matrone, 1976) and the proportion of amino acids directly incorporated in the microbial protein (Gawthorne & Nader, 1976) may also influence the availability of S to the Cu - Mo - S interaction. Suttle (1974c, 1975b) proved with the use of the depletion-repletion technique and semi-purified diets that S in both the organic and inorganic forms was equally effective in the Cu - S and the Cu - Mo - S interactions respectively. Contrary to Suttle's results, Goodrich & Tillman (1966) and Huisingh & Matrone (1976) observed different responses in the interactions of S with Cu and Cu - Mo depending on the form in which S was supplied. Since so many factors can influence the availability of S, it seems unlikely

that fixed Cu : Mo ratios or formulae can be devised for use in animal nutrition to suit all circumstances.

The low hepatic Cu retention observed at the low level of S intake in Trial 3 is probably best explained by the Cu - Mo - S interaction in the rumen. The negative hepatic Cu retentions observed at the S intakes of 4 and 5,3 g /sheep/day indicate that the Cu - Mo - S interaction may also occur at the body tissue level. Elevated plasma Cu levels, mainly due to an increase in the direct reacting Cu fraction of plasma, were considered by Suttle (1974b) to be a true systemic effect of the Cu - Mo - S interaction. High kidney Cu levels and increased rates of Cu excretion through the urine were considered to be the result of the high concentration of the direct reacting Cu fraction in plasma (Suttle, 1974b). They may also be considered as systemic expressions of the Cu - Mo - S interaction. It is clear, therefore, that the reactions taking place between Cu, Mo and S can occur both in the digestive tract of the sheep and at cellular level, as suggested by Dick et al. (1975). The supplementation of S during Trial 3 resulted in increased plasma and kidney cortex Cu levels typical of the so-called systemic effects of Cu - Mo - S interaction. The very high positive correlation between Cu and Mo concentrations in the kidney cortex for all treatments which received additional S may, therefore, also be considered as a true systemic effect of this interaction.

The relatively high plasma Cu levels during the first 42 days of Trial 3, even before any additional S was provided, might be considered as an expression of this systemic effect of the Cu - Mo - S interaction. These high Cu levels were observed in all sheep at the onset of the trial. Furthermore, none of the other systemic effects, viz. elevated kidney Cu levels or a high correlation between kidney Cu and Mo was observed in the Initial slaughter treatment group. It may be concluded, therefore, that these systemic effects were not detectable at the S intake level of 2,88 g /sheep/day (2,7 g S/kg DM) during the first 42 days of the trial nor in the case of the

Low S treatment during the remainder of the trial. Smith & Wright (1975b) suggested that changes in plasma Cu levels are elicited only above a critical dietary Mo concentration. From the present trial it appears that critical dietary S levels may also be necessary before systemic effects due to the Cu - Mo interaction can be expected. At a daily intake of 58 mg Cu and 35 mg Mo per sheep, the systemic effects were observed when the S intake increased from 2,88 g (0,27%) to 3,86 g (0,38%)/sheep/day. At concentrations of 58 mg Cu and 2,7 g S/kg DM, elevated plasma and kidney Cu levels were observed in Trial 1 at Mo levels of 45 mg/kg DM.

Elevated plasma Cu and direct reacting Cu levels were reported by Smith et al. (1968) at levels of 25 mg Mo and 1,8 g S/kg diet, by Suttle & Field (1968) at Mo and S concentrations of 50 mg and 3,6 g/kg feed respectively, and by Bingley (1974) at 120 mg Mo and 2,5 g S/sheep/day. At a Mo intake of 12 mg/day Bingley (1974) still observed slightly elevated plasma Cu levels. Smith & Wright (1975b) observed increased plasma Cu levels at Mo intakes of between 8 and 16 mg/day. Marcilese et al. (1970) reported increased kidney and urine Cu levels on diets containing 50 mg Mo and 1,3 g S/kg feed. Dick (1956) found an increase in plasma Cu levels at different stages after the onset of his trials, depending on Mo and S intakes. At the high Mo, high S intakes, plasma Cu increased immediately, while at lower levels of Mo and/or S these increases appeared later in the trial.

Widely different levels of both Mo and S are therefore apparently effective in promoting the systemic Cu - Mo - S interaction. An important contributing factor may be that semi-purified diets were used in most of the reported experiments where all the S was supplied as sulphate, while natural feeds were used in the present trial. Differences in S metabolism in the rumen have been reported to depend on the source of S (Huisingh & Matrone, 1976). Relatively high dietary levels of Mo and S are required to reduce the Cu content of the liver (Harker, 1976). It was observed that hepatic Cu

concentrations can be maintained (Ross, 1970) and that sheep can die from Cu toxicity months after the withdrawal of all additional Cu from their diets (Bracewell, 1958; Barden & Robertson, 1962). The reduction of the hepatic Cu content is therefore essential if Cu accumulation has already taken place in the liver. This could be achieved by exploiting the systemic interaction between Cu, Mo and S.

An atomic ratio between Cu and Mo of 2 : 1 was observed by Bremner & Young (1978) in the kidneys of sheep receiving additional S above a low S intake. Under similar conditions a ratio of 2,15 : 1 was observed in the present trial. Smith & Wright (1975b) observed an average Cu : Mo ratio of 1,7 : 1 (varying between 2 : 1 and 3 : 3) in the TCA insoluble fraction of plasma of sheep receiving additional dietary Mo and S. These ratios were considered as evidence for the presence of compounds containing Cu and Mo at fixed ratios and unavailable to the body. However, to observe such fixed ratios, the presence of Cu and Mo in other forms in the kidneys should be low.

The inclusion of S in the ration in this trial resulted not only in the high positive correlation between Cu and Mo in the kidney cortex but also in a significant reduction in the Mo levels of the plasma, spleen, muscle and testes. This finding is in accord with the results of Dick (1956), Huisingh et al. (1973) and Suttle (1975b). It is suggested that S could have exerted a similar depleting effect on the Mo in the kidneys, while simultaneous deposition of Mo, in conjunction with Cu and in a form unavailable to the body, was occurring in the kidneys. Evidence of this is the fact that even though the cortex Mo levels during Trial 3 were more or less the same for all treatments, the high correlation between Mo and Cu existed only when the S intakes were high, i.e. when the other so-called system effects were observed. Dick (1956) reported decreased Mo concentrations in all organs after the addition of dietary S, with the exception of a slight increase in the kidneys. Bremner & Young (1978), on the other hand, observed a drop in kidney Mo concentration with the feeding of extra S. These differences may be explained by the extent to which the Cu-Mo

complex accumulated in the kidneys relative to the original renal Mo concentration.

The first increment of additional S resulted in a significant reduction in Mo levels in the tissues during the trial while the second increment did not cause further reductions in tissue Mo levels. A similar non-linear effect of S supplementation on Mo absorption and Mo concentrations was observed in blood (Dick, 1956) and in plasma and urine (Grace & Suttle, 1979). During Trial 3 of the present study the second increment of S exerted no further effect on Mo concentration in the tissues, though the Cu and Mo levels in the kidneys increased significantly above the levels measured at the medium S treatment. This might lend support to the suggestion that the effect of S on Mo concentration in tissues functioned independently of the so-called systemic effect, resulting therefore, in a correlation between the Cu and Mo in the kidneys.

CHAPTER 7

TRIAL 4: THE EFFECT OF DIFFERENT LEVELS OF DIETARY MO ON CU AND MO METABOLISM IN SHEEP

INTRODUCTION

It was suggested in Trial 3 that relatively high levels of dietary Mo and S would be required to reduce the level of accumulated Cu in the liver. Dick (1954) measured no changes in hepatic Cu content at Mo intakes of between 20 and 100 mg/sheep/day. It may be concluded from Dick's results that dietary Mo intakes in the region of 20 mg/sheep/day or 20 mg Mo/kg feed should be optimum and sufficient to control hepatic Cu accumulation. These observations by Dick (1954) did not agree with results obtained in cattle receiving diets containing up to 200 mg Mo/kg DM (Vanderveen & Keener, 1964; Huber et al., 1971).

If fairly high levels of Mo and S are used to reduce hepatic Cu content, a significant build-up of Cu and Mo may take place in the kidneys, as observed in Trials 1 and 3. Van Adrichem (1965) mentioned the possibility of kidney damage when high levels of Mo and SO_4^{2-} are fed to sheep for prolonged periods. On the other hand, Bingley (1974) stated that sheep tolerated high doses of Mo and SO_4^{2-} for protracted periods.

A trial was therefore designed in which sheep received relatively high levels of Cu and S and different levels of Mo. The distribution of Cu and Mo in the body was followed under these conditions. Clinical and physiological tests on the liver and kidneys were also carried out to establish the degree of tissue damage in these organs due to high Cu and Mo intakes. These investigations are reported in Chapter 8.

PROCEDURE

Experimental animals, treatments and procedure

Forty S.A. Mutton Merinos (20 wethers and 20 ewes), approximately one year of age, were allocated to five groups, as described in Chapter 2. All the groups received the basic ration high in Cu and without additional Mo, during a pre-experimental period of 42 days. At the end of this stage, one group, called the pre-experimental slaughter group (Pr-X) was slaughtered, while the other groups were fed different levels of Mo in their rations and were called the No Mo, Low Mo, Medium Mo and High Mo treatments.

The sheep received a ration consisting of veld hay and a concentrate mixture comprising 35,5% maize meal, 29,9% sunflower oil cake meal, 29,9% sterilized poultry manure (trade name, K3), 3% urea, 1,5% sodium sulphate and 0,0955% cupric sulphate. The general experimental routine followed, was the same as that described in Chapter 2, and the trial lasted for 193 days.

The following analyses were performed during this trial:

a) feeds - Mo, Cu, Zn, Fe, Mn, S, Ca, P and crude protein;

b) whole blood - PCV, haemoglobin and red blood cell count;

c) serum - GOT, LDH, Ca, P, Mg, Zn, Fe, K, total protein and albumin;

d) plasma - Mo, total Cu, direct reacting Cu, arginase;

e) livers, kidneys (medulla and cortex), spleens, lungs, 1. dorsi muscle, heart muscle and wool - Mo, Cu, Fe and Zn.

A kidney clearance study, using inulin, endogenous urea and endogenous creatinine, was carried out on 14 of the ewes during the last two months of the trial. A histological examination of liver and kidney samples was done, as described in Chapter 2. Clinical investigations are discussed in Chapter 8.

RESULTS

Feed intakes and composition of ration

A sheep consumed an average of 274 g DM of the concentrate mixture per day and an average hay intake of 698 g DM/sheep/day was recorded. The average daily mineral intake per sheep during the experimental period and the concentration of minerals in the ration is presented in Table 7.1. The average Cu : Mo ratios in the rations were 3,9 for the Low Mo, 2,2 for the Medium Mo and 1,4 for the High Mo groups. An average Ca : P ratio of 2,4 : 1 was measured in the feed. The average crude protein intake per sheep was 122 g/day during the trial.

Body and organ mass

Differences in final mass and body mass changes between treatments were small and not statistically significant. The organs of the pre-experimental group were significantly lighter than the organs at the end of the trial (Table 7.2) No differences in organ mass due to treatment were observed at the end of the trial. The DM percentages of the organs of all animals remained remarkably constant, except for the low liver DM percentage of a ewe in the Low Mo treatment which died of cardiovascular shock during the kidney clearance study.

Clinical investigation

One sheep from the High Mo and one from the Medium Mo groups developed diarrhoea on the second day after the introduction of Mo to the diets. This diarrhoea lasted for two days. No further incidence of diarrhoea was observed in any sheep for the rest of the trial. During the kidney clearance study, one ewe (Low Mo) died, apparently due to cardiovascular shock.

Table 7.1 Total mineral intakes/sheep/day and average concentration of minerals in experimental rations (DM basis)

Treatments	Mo mg	Cu mg	Zn mg	Fe mg	Mn mg	S g	Ca g	P g
	Daily intake of minerals/sheep							
No Mo	0,6	78,5	59	302	236	3,69	10,3	4,35
Low Mo	20,8	81,5	60	298	236	3,72	10,1	4,32
Med Mo	38,4	84,2	60	300	236	3,85	10,2	4,32
High Mo	58,5	82,6	59	303	236	3,80	10,2	4,30
	Concentration of minerals							
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	%	%
No Mo	0,6	80,8	61	311	243	0,38	1,06	0,45
Low Mo	21,4	84,0	62	307	244	0,38	1,04	0,45
Med Mo	39,6	86,8	62	310	243	0,40	1,05	0,45
High Mo	60,3	85,2	61	312	243	0,39	1,05	0,44

Table 7.2 Final body mass and body mass increases, mass of fresh organs and percentage DM in organs (± SE of means)

	Treatments***				
	Pr - X	No Mo	Low Mo	Med Mo	High Mo
Final body mass (kg)	-	33,9	35,1	34,8	33,4
Mass increase (kg)**	-	11,8	12,9	12,5	11,7
Liver : Fresh (g)	331[a]	386[b]	393[b]	397[b]	383[b]
DM (%)	29,6 ± 0,37	29,4 ± 0,45	30,1 ± 0,50*	30,7 ± 0,11	30,8 ± 0,21
Spleen : Fresh (g)	42,5[a]	71,4[b]	71,6[b]	69,5[b]	73,8[b]
DM (%)	22,2 ± 0,21	21,4 ± 0,54	21,8 ± 0,22	21,9 ± 0,27	21,8 ± 0,12
Lungs : Fresh (g)	271[a]	315[b]	330[b]	321[b]	329[b]
DM (%)	19,2 ± 0,33	20,5 ± 0,24	19,9 ± 0,26	20,6 ± 0,20	20,3 ± 0,36

* One sheep died of cardiovascular shock. Its liver contained 22,4% DM and was excluded from calculations.

** Including the 42 days of the pre-experimental period.

*** Averages within organs with different superscripts a - b, denote differences at $P < 0,01$ level of significance.

Organs and tissues

There appeared to be no trend relating the sex of the sheep to accumulation of minerals in the organs, therefore data from both sexes were pooled in the analyses.

a) Copper

The addition of Mo up to a level of 38 mg/sheep/day resulted in marked reductions in liver Cu content (Table 7.3). Despite wide variations in the Cu concentration of the livers within treatments, differences between the No Mo and Medium Mo were statistically significant. However, at a Mo intake of 58 mg/sheep/day elevated Cu levels were again observed to be almost as high as the liver Cu levels of the group receiving no Mo (Figure 7.1). In all the other organs and tissues analysed, no change in Cu concentrations was measured up to the level of 38 mg Mo/kg/day, except in the kidneys. A slight increase in Cu also occurred in the heart muscle. This trend of Cu accumulation was obvious in all the tissues at the 58 mg level of Mo intake and very pronounced in the kidneys, especially the kidney cortex (Figure 7.2).

During the experimental period of 193 days, 0,35% of the dietary Cu accumulated in the livers of the sheep receiving no Mo. This estimate is arrived at after taking into account the liver Cu content of the pre-experimental group after 42 days on the high Cu ration.

b) Molybdenum

Any increase in level of Mo supplemented to the sheep was reflected in the concentrations of Mo in the organs and tissues (Table 7.4). An increase of approximately 20 mg Mo in the diet of a sheep per day was sufficiently high to cause a statistically significant increase ($P < 0,01$) in the Mo concentration of the tissues. The most dramatic increase in Mo concentration was recorded in the kidney cortex. In this respect, Cu and Mo behaved similarly (Figure 7.2).

Table 7.3 The influence of different levels of Mo intake on the content and concentration of Cu in the tissues and wool of sheep (± SE of mean)

	Copper					
Treatments	Pr-X	No Mo	Low Mo	Med Mo	High Mo	
Number	1	2	3	4	5	F-test**
mg Mo/day	0	0,6	20,8	38,4	58,5	
Tissues						
Liver : mg/kg DM	608 ± 48	987 ± 80	812 ± 58	660 ± 62	835 ± 63	4<2 1<2,5*
mg	59,8 ± 8	112,4 ± 10	93,3 ± 5	79,4 ± 9	95,6 ± 6	1<2,3,5 4<2*
Kidneys: Cortex: mg/kg DM	16 ± 0,8	26 ± 1,9	26 ± 2,3	94 ±15,2	236 ± 36,6	5>4>3,2,1
Medulla : mg/kg DM	-	8,2 ± 0,9	8,4 ± 0,3	14,9 ± 1,5	40,4 ± 4,4	5>4>3,2
Lungs: mg/kg DM	13,0 ± 0,8	11,5 ± 0,6	11,5 ± 0,4	12,5 ± 0,5	17,4 ± 0,9	5>4,3,2,1
Spleen : mg/kg DM	3,7 ± 0,4	2,2 ± 0,3	4,1 ± 0,4	3,7 ± 0,5	13,0 ± 1,8	5>4,3,2,1 2<4*,3,1*
Muscle (fat-free) : L dorsi: mg/kg DM	6,2 ± 0,5	5,0 ± 0,3	4,6 ± 0,3	5,6 ± 0,4	6,1 ± 0,2	5>3,2* 1>3*
Heart : mg/kg DM	19,4 ± 0,5	18,8 ± 0,9	18,8 ± 0,6	22,6 ± 0,6	27,4 ± 0,8	5>4*>3,2,1
Wool (fat-free) : mg/kg DM	-	6,5 ± 0,6	6,0 ± 0,5	6,8 ± 0,9	5,7 ± 0,3	NS

* Differences significant at P<0,05 and

** Differences significant at P<0,01 levels of significance

NS Statistically not significant.

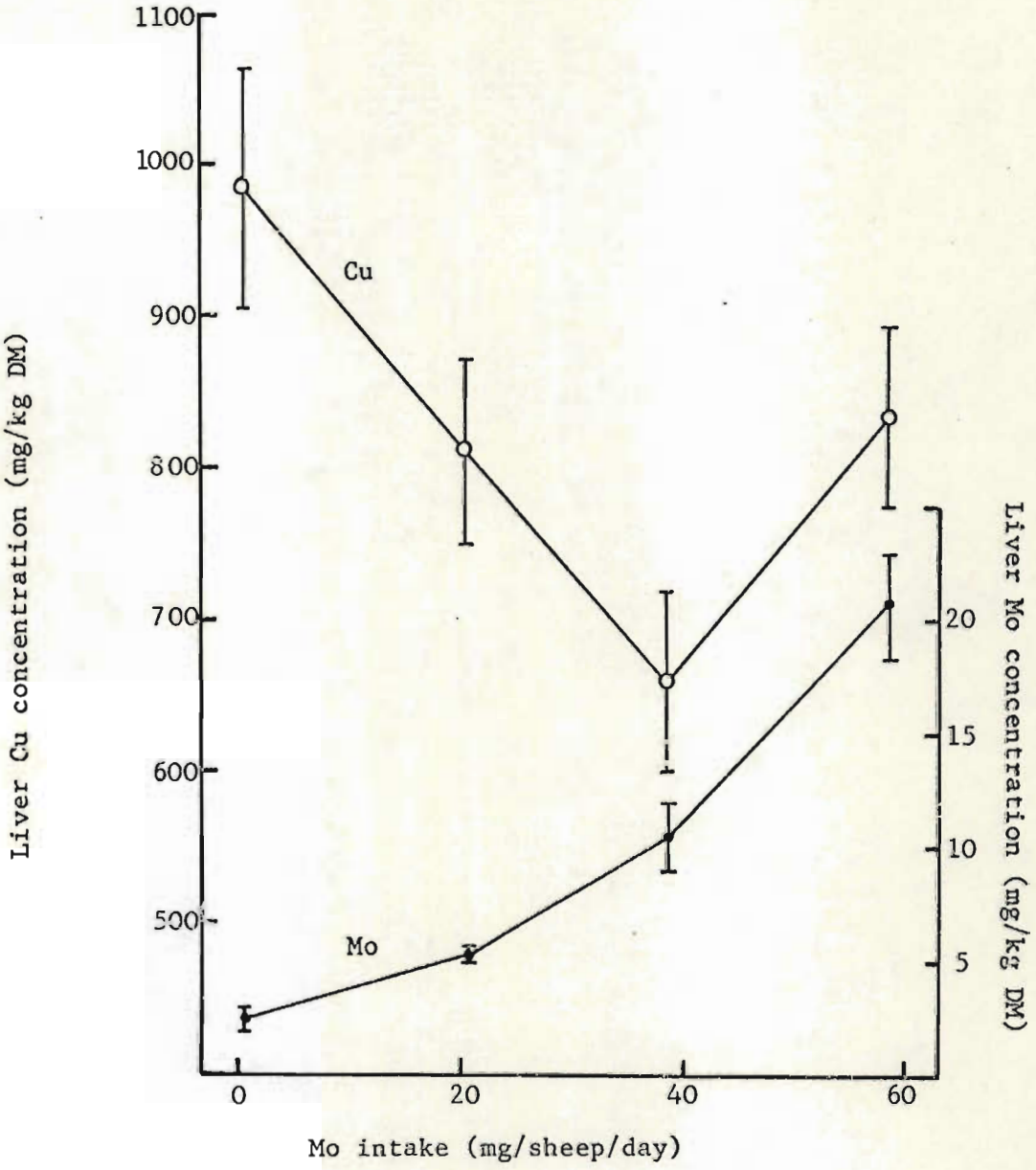

Fig. 7.1 Influence of Mo intake/sheep/day on liver Cu and Mo concentrations.

Vertical bars represent SE of means

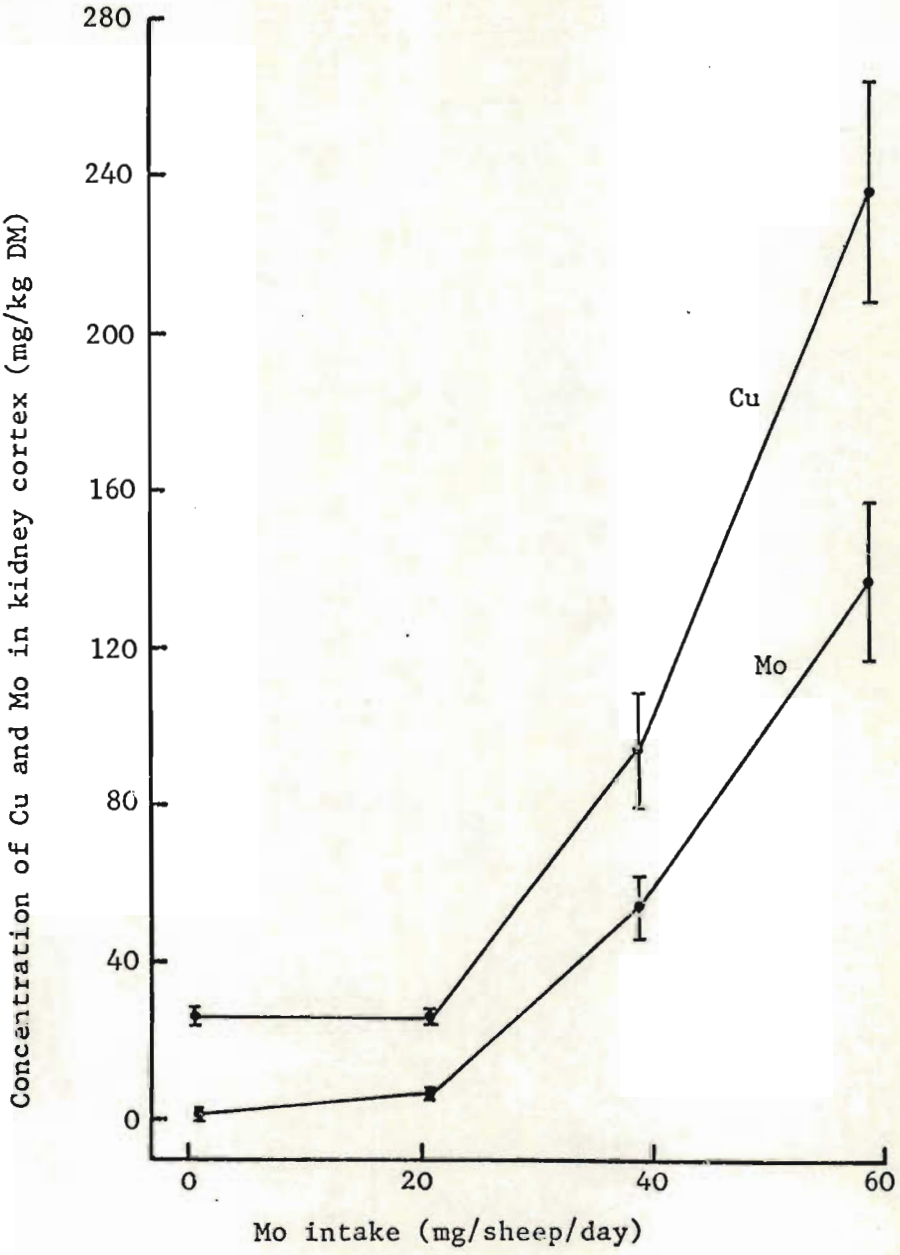

Fig. 7.2 Influence of Mo intake/sheep/day on the concentrations of Cu and Mo in the kidney cortex.

Vertical bars represent SE of means

Table 7.4 The influence of different levels of Mo intake on the content and concentration of Mo in the tissues and wool of sheep (± SE of mean)

	Molybdenum					
Treatments	Pr-X	No Mo	Low Mo	Med Mo	High Mo	
Number	1	2	3	4	5	F-test**
mg Mo/day	0	0,6	20,8	38,4	58,5	
Tissues						
Liver:						
mg/kg DM	2,9 ± 0,2	2,5 ± 0,4	5,6 ± 0,3	11,0 ± 1,4	21,2 ± 2,5	5>4>3>2,1
mg	0,28 ± 0,04	0,31 ± 0,05	0,67 ± 0,06	1,26 ± 0,11	2,48 ± 0,33	5>4>3>2,1
Kidneys:						
cortex: mg/kg DM	1,5 ± 0,1	1,7 ± 0,1	6,5 ± 0,7	54,7 ± 8,6	136,5 ± 20,5	5>4>3>2,1
medulla: mg/kg DM	-	1,4 ± 0,2	2,3 ± 0,4	7,1 ± 0,9	24,1 ± 3,1	5>4>3>2
Lungs: mg/kg DM	0,19 ± 0,07	0,58 ± 0,03	1,23 ± 0,10	2.39 ± 0,16	6,25 ± 0,55	5>4>3>2>1
Spleen: mg/kg DM	0,49 ± 0,02	0,24 ± 0,03	0,84 ± 0,19	3,42 ± 0,36	11,28 ± 1,30	5>4>3>2,1
Muscle (fat-free):						
L dorsi: mg/kg DM	0,20 ± 0,10	0,18 ± 0,07	0,15 ± 0,07	0,44 ± 0,07	0,94 ± 0,15	5>4>3,2,1
Heart: mg/kg DM	0,12 ± 0,05	0,05 ± 0,02	0,43 ± 0,06	1,22 ± 0,37	1,74 ± 0,55	5,4>3>2,1
Wool (fat-free): mg/kg DM	-	0,38 ± 0,15	0,37 ± 0,10	0,45 ± 0,15	1,01 ± 0,09	5>4,3,2

** Differences significant at $P<0,01$ level of significance

c) Copper to molybdenum ratios

High positive correlations were observed between Cu and Mo concentrations of the kidney cortices of the groups receiving additional Mo (Table 7.5). Similarly high correlations in Cu : Mo of the medulla were observed at the two highest Mo levels.

d) Iron and zinc

None of the differences obtained in Fe or Zn concentration of the tissues was statistically significant (Table 7.6). Very wide variations in Fe concentrations of the organs were observed within treatments, rendering possible differences between treatments insignificant.

Blood analyses

a) Plasma Cu and Mo

Significant increases in total plasma Cu levels were obtained in the Medium and High Mo treatments above the Low and No Mo treatments (Table 7.7 and Figure 7.3). These differences seemed to be due mainly to the direct reacting Cu fraction of plasma because the differences between total and direct reacting Cu remained practically constant for all treatments (Figure 7.4).

The concentration of Mo in plasma followed the same pattern as the Mo concentrations in the other organs. Higher Mo intakes increased the Mo concentrations in plasma significantly ($P < 0,01$) between low and medium levels and further between medium and high Mo intakes.

b) Haematological parameters

Throughout the experiment the PCV percentages, haemoglobin levels and red blood cell counts remained relatively constant without any

Table 7.5 The influence of level of Mo intake on the Cu : Mo ratios in the kidneys

Treatments	Cortex		Medulla	
	Atomic ratio Cu : Mo	Correlation (r) Cu : Mo	Atomic ratio Cu : Mo	Correlation (r) Cu : Mo
Pr-X	10,67	0,03	-	-
No Mo	15,29	-0,02	5,86	-0,33
Low Mo	4,54	0,84	3,65	0,06
Med Mo	1,71	0,98	2,10	0,87
High Mo	1,73	0,92	1,66	0,88

Table 7.6 The influence of different levels of Mo on the concentration of Fe and Zn in the organs and the wool of sheep (± SE of mean)

		Zinc and Iron				
Treatment	Pre-exp	No Mo	Low Mo	Med Mo	High Mo	
Number	1	2	3	4	5	F-test*
mg Mo/day	0	0,6	20,8	38,4	58,5	
Tissues :						
Liver:						
mg Fe/kg DM	287 ± 28	281 ± 36	289 ± 25	363 ± 53	316 ± 44	NS
mg Zn/kg DM	122 ± 4	120 ± 6	112 ± 3	111 ± 4	117 ± 4	NS
Kidney :						
cortex:						
mg Fe/kg DM	181 ± 14	230 ± 42	256 ± 28	248 ± 40	303 ± 71	NS
mg Zn/kg DM	141 ± 19	154 ± 16	148 ± 14	149 ± 16	156 ± 17	NS
medulla:						
mg Fe/kg DM	-	78 ± 14	96 ± 15	106 ± 10	113 ± 17	NS
mg Zn/kg DM	-	88 ± 6	90 ± 5	106 ± 9	82 ± 17	NS
Lungs :						
mg Fe/kg DM	375 ± 50	487 ± 46	484 ± 42	439 ± 57	449 ± 39	NS
mg Zn/kg DM	63 ± 3	75 ± 2	83 ± 2	78 ± 3	76 ± 2	NS
Spleen :						
mg Fe/kg DM	574 ± 33	699 ± 25	754 ± 39	566 ± 40	622 ± 45	NS
mg Zn/kg DM	102 ± 5	93 ± 2	91 ± 2	99 ± 2	100 ± 2	NS
Muscle (fat-free) :						
L. dorsi :						
mg Fe/kg DM	77 ± 5	87 ± 15	65 ± 5	68 ± 4	65 ± 4	NS
mg Zn/kg DM	82 ± 2	81 ± 2	82 ± 3	89 ± 5	80 ± 2	NS
Heart :						
mg Fe/kg DM	239 ± 6	235 ± 15	201 ± 12	231 ± 6	238 ± 12	NS
mg Zn/kg DM	77 ± 2	73 ± 1	76 ± 2	79 ± 3	78 ± 2	NS
Wool (fat free) :						
mg Fe/kg DM	-	16 ± 9	24 ± 8	9 ± 4	8 ± 6	NS
mg Zn/kg DM	-	111 ± 4	110 ± 1	106 ± 3	110 ± 3	NS

*NS - Statistically not significant

Table 7.7 The influence of different levels of Mo on the average concentrations of total plasma Cu, direct reacting Cu (DR Cu) and total plasma Mo.

	Plasma Cu and Mo (mg/dm^3)				
Treatments	No Mo	Low Mo	Med Mo	High Mo	
Number	2	3	4	5	F-test**
mg Mo/day	0,6	20,8	38,4	58,5	
Copper :					
Pr-X Period					
Total Cu	0,90	0,90	0,98	0,92	NS
Exper. Period					
Total Cu	0,95	0,96	1,25	1,65	5>4>3,2
DR Cu	0,22	0,27	0,59	0,97	5>4>3,2
Difference	0,73	0,69	0,66	0,68	NS
Molybdenum :					
Pr-X Period	0,13	0,12	0,12	0,11	NS
Exper. Period	0,06	0,12	0,54	0,92	5>4>3,2

** Differences significant at $P<0,01$

NS Not significant

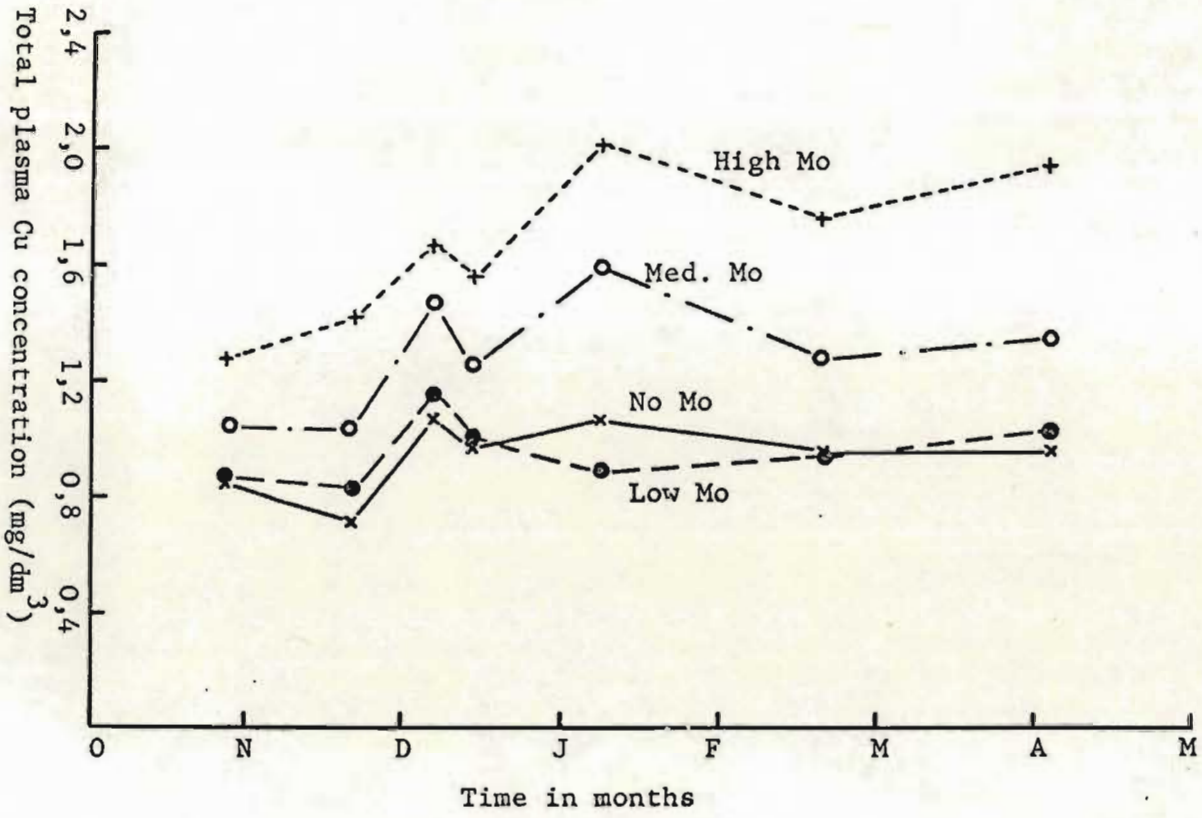

Fig. 7.3 The effect of different levels of dietary Mo on the concentration of total plasma Cu

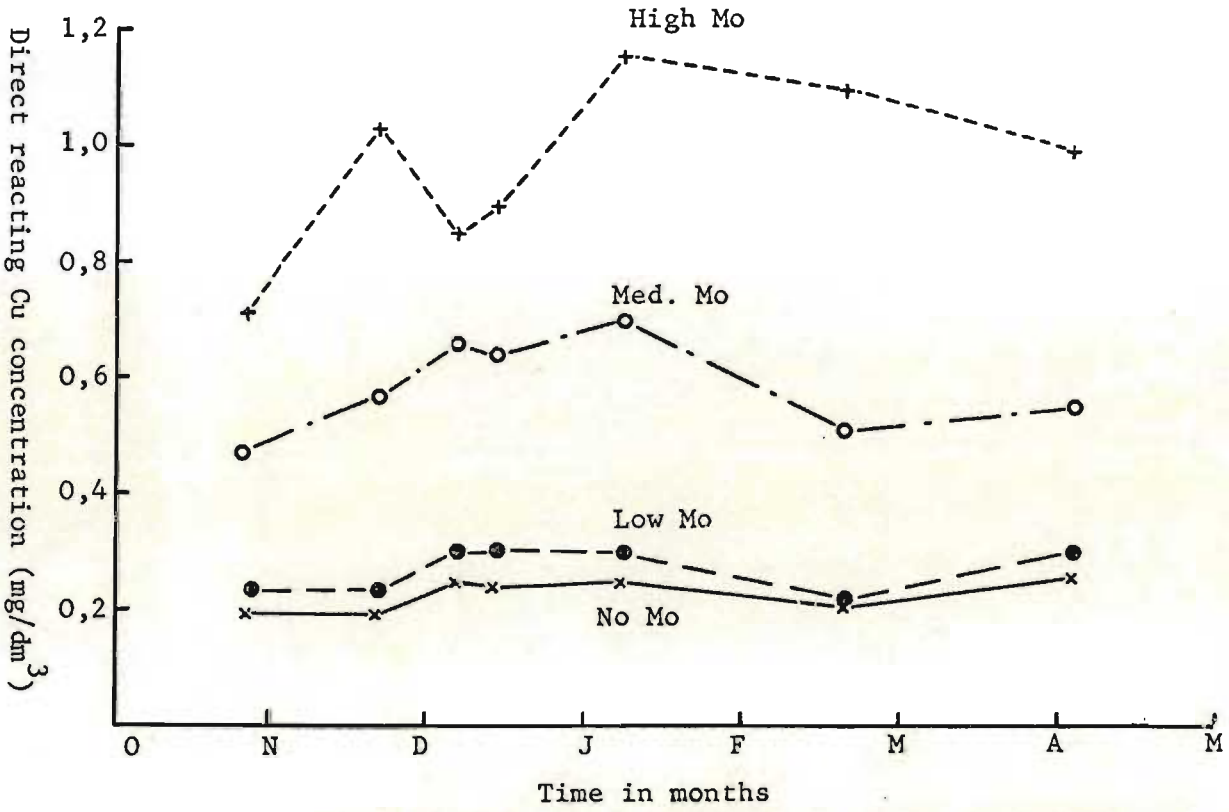

Fig. 7.4 The effect of different levels of dietary Mo on the concentration of direct reacting Cu in plasma.

significant differences between treatments. The average values of all the readings are presented in Table 7.8, together with the SE of the means.

c) Protein fractions and other minerals in serum

The mineral and protein concentrations in the serum were determined on four occasions during the trial. These values remained practically constant in all analyses, therefore average values are presented in Table 7.9. The only exception was the Fe levels which fluctuated quite widely between different collections but not between treatments at a specific collection.

None of the minerals or the protein fractions in the serum showed any significant differences related to Mo treatments. The phosphorus concentration in the serum was substantially higher than the optimum level suggested by the Allerton laboratories. All other mineral levels and the protein fractions corresponded to those suggested as optimum levels.

DISCUSSION

The depressing effect of Mo plus S on Cu retentions in the livers of sheep is well documented. However, there are only few investigations in which the effect of more than two levels of dietary Mo on hepatic Cu content have been tested and which were free of confounding effects of other treatments. Dick (1954) found a decrease in hepatic Cu content up to an intake of 20 mg Mo/sheep/day. Between 20 and 100 mg Mo, Dick (1954) observed no further changes in the Cu content of the liver. Vanderveen & Keener (1964) and Huber et al. (1971) reported a progressive decrease in liver Cu concentration of dairy cattle at increasing levels of dietary Mo. At high Mo intakes (100 to 200 mg/kg feed) Vanderveen & Keener (1964) found very low and fluctuating levels of Cu in the liver.

Table 7.8 The effect of level of Mo feeding on average packed cell volumes (PCV), haemoglobin values and red blood cell (RBC) counts in blood (± SE of mean)

Treatments	PCV %	Haemoglobin g/100 cm^3	RBC count x 10^6
No Mo	29,9 ± 0,48	11,5 ± 0,21	8,98 ± 0,36
Low Mo	27,8 ± 0,54	10,7 ± 0,24	8,24 ± 0,29
Med Mo	27,2 ± 0,51	10,5 ± 0,23	8,86 ± 0,29
High Mo	29,4 ± 0,46	11,0 ± 0,18	8,39 ± 0,26

Table 7.9 Average concentrations of minerals and protein fractions in serum at different levels of Mo intakes.

	Treatments*				
Minerals	No Mo	Low Mo	Med Mo	High Mo	Optimum level**
Ca (mg/dm^3)	95	98	96	99	100
Inorg.P (mg/dm^3)	74	74	73	73	42
Mg (mg/dm^3)	21	21	21	21	26
Zn (mg/dm^3)	1,13	1,18	1,01	1,05	0,98
Fe (mg/dm^3)	1,85	1,84	1,88	1,72	1,87
K (mg/dm^3)	178	174	176	174	191
Proteins					
Tot.prot. ($g/100cm^3$)	6,1	6,0	6,2	6,3	6,99
Albumin ($g/100cm^3$)	3,6	3,4	3,4	3,7	3,03

* Differences between treatments were statistically not significant

** Allerton veterinary laboratories

In the present trial an increase in Mo intake up to 38 mg Mo/sheep/day caused a decrease in the liver Cu content. With a further addition of Mo (59 mg Mo/day) an increase in liver Cu content was observed. This response was contrary to expectations. At the lower Mo intakes, no changes in Cu concentration of the other tissues, except the liver and kidneys, were noticed. With an Mo intake of 59 mg Mo/sheep/day increases in the Cu concentrations were observed in all the tissues, except in wool. This effect was quite dramatic in the kidneys, especially in the renal cortex, and was already evident at an intake of 38 mg Mo/day.

Elevated renal and plasma Cu levels are well recognised consequences of the Cu - Mo - S interaction in the body of the ruminant, and were observed in Trials 1 and 3, and by various other authors. These elevated Cu levels in the kidneys and plasma have been suggested to be the result of the accumulation of Cu : Mo - containing compounds in these tissues in forms unavailable to the body. High correlations between Cu and Mo in fractions of the plasma and in the kidneys were observed by Bremner & Young (1978) and in Trial 3 of this study. Ratios between Cu and Mo in the kidneys in the present trial varied slightly from the ratios reported, being generally slightly lower.

From the results of the present trial it can be concluded that complexes containing Cu and Mo tended to accumulate, firstly in the kidneys, but eventually also in the liver and in other organs when high dietary levels of Cu, Mo and S were fed to sheep. In Trial 1, elevated Cu levels in the spleens were observed at high Mo intakes, an observation similar to the results of the present trial. Although no verification of the results of this trial could be found in the literature, the concept that Cu and Mo could be present in unavailable forms in the body seems to be accepted (Cook et al., 1966); Ward, 1978). Suttle (1974b) concluded from work on non-ruminants, that dietary Mo concentrations below 100 mg/kg feed would decrease the Cu status of the animal, but above 100 mg Mo/kg feed "the more familiar response namely increased tissue Cu concentrations accompanied by (symptoms of) clinical Cu deficiency, pertains".

In non-ruminants, elevated Cu concentrations in the tissues, especially the liver, were observed at high Mo intakes (Miller, Price & Engel, 1956; Mills, 1960; Arthur, 1965; Dale, Ewan, Speer & Zimmerman, 1973). Mills (1960) pointed out that additional SO_4^{2-} reduced these elevated liver Cu levels in rats, in contrast to the effect of SO_4^{2-} on the Mo - Cu interaction in ruminants. The formation of compounds containing Cu and Mo at a ratio of 1,5 : 1 was observed by Mills & Mitchell (1971) in liver tissue of rats receiving high levels of Cu and Mo. Suttle (1974b) mentioned the possibility that high levels of Mo in the bloodstream and tissue tended to favour the formation of inorganic and organic Cu - Mo complexes, which could then accumulate in the tissues.

With the use of radio-isotope studies, Fisher & Clawson (1976) observed a reduced urinary Cu excretion in ruminants receiving high levels of dietary Mo, but increased faecal Cu excretions. They suggested that this was the result of an increased biliary excretion of organically bound Cu, after uptake of this Cu by the liver. Contrary to this, Grace & Suttle (1979) concluded from evidence in their trials on sheep that poorly exchangeable Mo complexes, formed at high Mo plus S intakes, were ineffectively excreted by both the urinary and faecal routes. Grace & Suttle (1979) suggested the formation of Cu containing thiomolybdates in ruminants as the cause for this poor excretion of Cu and Mo. This difference in interpretation of results awaits final clarification.

In order to ascertain the reason for the lack of support in the literature for the results observed in the present trial (Trial 4), comparisons have to be made between this trial and others in the literature. Suttle (1974b) pointed out that high Mo levels (100 to 1000 mg kg feed) are usually used in experiments with non-ruminants while Mo levels of below 100 mg/kg feed are offered to ruminants. However, in the present trial the accumulation of Cu in the tissues was observed at 60 mg Mo/kg DM of the ration. It is proposed that the accumulation of Cu in tissues can occur in ruminants at an Mo

level of intake as low as 60 mg/kg feed, provided sufficient dietary Cu and S are available. However, if Cu levels are only measured in the liver, kidney and plasma and at only two levels of Mo supplementation (Ross, 1964, 1966, 1970; Van Adrichem, 1965; Hogan, Ris & Hutchinson, 1966; Marcilese et al., 1969; Van der Berg & Van der Schee, 1973; Bremner & Young, 1978), the accumulation of Cu in tissues, except the kidneys and plasma, may not be observed. Any accumulation of bound Cu in the liver will not be noticeable due to a decrease in liver Cu content owing to Mo supplementation which may be more rapid than the Cu accumulation. The development of a state of Cu deficiency due to Mo may also result in a lack of Cu in the body to bind with Mo, and limited accumulation of Cu can be expected. In the present trial no sign of Cu deficiency was observed in any of the haematological parameters or in the Cu content of the liver or wool. However, tissue Cu concentrations, eg. liver Cu levels, will become unreliable indicators of the Cu status of the animal at high Mo intakes if Cu accumulates in these organs in unavailable forms.

Dick (1954) observed constant hepatic Cu retentions at Mo intakes above 20 mg/sheep/day; this is contrary to the decreases in liver Cu content observed at similar Mo levels in the present trial. It is possible that S was a limiting factor in Dick's work at an intake of 0,46 g/sheep/day. Vanderveen & Keener (1966) and Huber et al. (1971) reported signs of Cu deficiency in their trials at high Mo intakes. Bingley (1974) observed slight increases in liver Cu levels at high Mo intakes. However, these last-mentioned results are unexpected in view of the fact that the sheep received 8 to 10 mg Cu/day and 120 mg Mo and 5 g SO_4^{2-} for 29 months.

The Mo concentrations of the tissues in the present trial followed a pattern of accumulation which corresponded to the level of dietary Mo intake/day. These observations agreed with those in Trial 2 and with those reported by Cunningham & Hogan (1959) and Lesperance & Bohman (1963). It was shown in Trial 3 that tissue Mo levels also depend on the S intake, and that a constant Mo level per tissue can

be expected above a certain level of S in the diet. Accepting that the S intake during the present trial was above this minimum level as observed in Trial 3, it may be concluded that the concentration of the Mo in the tissues still depends on level of dietary Mo.

Whether the Mo is present in an available form to the body or not, is not clear. It would appear from the conclusions drawn in Trial 3 that most of the Mo in the kidneys was bound to Cu. At high concentrations of the Cu - Mo complex, eg. in the kidneys, a close relationship between Cu and Mo may be expected. However, at lower concentrations of this complex, as observed in the other tissues, the presence especially of Cu in other forms, will influence any ratios between Cu and Mo when determined on the complete organs.

After the commencement of Mo feeding in this present trial, two sheep had diarrhoea for a few days. Severe diarrhoea was observed by Suttle & Field (1968) in sheep receiving 50 mg Mo and 10 g SO_4^{2-}/kg feed. Diarrhoea as a sign of molybdenosis is usually observed only in cattle fed high Mo intakes (Cunningham & Hogan, 1959; Underwood, 1977; Ward, 1978). A reduction in plasma protein was suggested by Smith and Wright (1975b) as indicating that Mo supplementation affected protein metabolism. No differences in plasma protein or albumin were observed in the present trial. Bremner & Young (1978) suggested that the accumulation of Mo in the tissues and plasma had a toxic effect and was the reason for reduced growth rates observed in sheep receiving additional Mo. They arrived at this conclusion because additional S reduced the Mo levels in the tissues and improved the growth rate. However, it seems more likely that the additional Mo used by Bremner & Young (1978) reduced the availability of S to the micro-organisms in the rumen as observed by Huisingh & Matrone (1976) and others. Additional S would, therefore, alleviate this S deficiency to the micro-organisms, and better growth would result.

Elevated inorganic P levels in serum may point to a potential problem, namely urolithiasis in sheep (Pope, 1975). In Trial 4, at a concentration of 0,45% P in the ration and a Ca : P ratio of 2,4 : 1 urolithiasis should not be a serious risk. An interesting alternative reason for the elevated inorganic P levels in serum during this trial may be the presence of high levels of S and Cu in the diet. Goodrich & Tillman (1966) reported a depletion of P stores in the body at high levels of dietary S in the presence of Cu.

In the group on high dietary Cu levels without additional Mo, a relatively low proportion of dietary Cu (0,35%) accumulated in the liver. This is in agreement with the observations in the previous trials, where possible explanations for these low Cu retentions were discussed. However, the exceptionally high Cu content of livers in the pre-experimental group after 42 days on the high Cu diet (viz. 608 mg/kg DM), may have contributed to this low hepatic Cu retention observed in the No Mo group.

In Trial 3 it was proposed that minimum S and Mo levels in the diet are required before the so-called systemic effects of the Cu - Mo - S interaction will be observed. In this present trial a dietary Mo level of above 21 mg Mo/sheep/day was necessary to obtain the systemic effects, as compared to between 8 and 16 mg Mo/sheep/day observed by Smith & Wright (1975b). However, no negative Cu retentions were observed in the present trial at the Mo and S intakes of 38 mg and 3,9 g respectively, which corresponded well with the Medium S treatment in Trial 3 where a slight positive Cu retention was also observed.

CHAPTER 8

THE ANATOMICAL AND PHYSIOLOGICAL SOUNDNESS OF LIVERS AND KIDNEYS OF SHEEP RECEIVING HIGH LEVELS OF CU AND S AND DIFFERENT LEVELS OF MO DURING TRIAL 4

PROCEDURE

All sheep received high levels of dietary Cu (± 84 mg Cu/kg DM), additional S (total S concentration ± 0,38%) and different levels of Mo, as described in Chapter 7. The treatment groups were called No Mo, Low Mo, Medium Mo and High Mo depending on levels of Mo fed.

Serum GOT and LDH levels were determined regularly throughout the trial. Although the standard kits used, were developed for testing human plasma enzymes, their use with sheep serum was considered to be sufficiently accurate to show up relative differences even though the estimates of enzyme activity might not be absolutely correct. No statistical analyses were done on these data because changes in enzyme concentrations took place in individual sheep within a group.

The kidney clearance study was done on 14 of the 16 ewes in the trial. One ewe (217) in the Low Mo group died, presumably of cardiovascular shock during infusion of inulin. Another ewe in the Medium Mo group was excluded from the study because on both occasions when attempts were made to infuse inulin it showed symptoms similar to the one that died. One run of clearances with four collection periods was performed per ewe, with the exception of ewe 223 in the No Mo group on which two runs of collections were done. The averages of the collections per run per ewe were calculated. Clearances of inulin, creatinine and urea were calculated according to the formula given in Chapter 2.

If one of the four clearances obtained, differed markedly from the others, that result was omitted and the average of the remainder was calculated. No statistical evaluation was attempted because of the limited nature of the data.

A veterinary surgeon examined the organs and carcasses immediately after slaughter. Samples of liver and kidneys were taken after slaughter for histological study. Histological changes in the liver and kidneys were rated as follows :

0 = none
1 = minimal
2 = moderate
3 = pronounced.

No statistical analyses were attempted on the results because the values of and differences between treatments depend on the relative value assigned to the subjectively evaluated changes observed in these organs.

The first four numbers per treatment group, for example 211, 212, 213 and 214 in the Low Mo treatment, were allocated to the wethers and the second four, eg. 215, 216, 217 and 218, to the ewes.

RESULTS

Macro-anatomical evaluation

Slight liver degeneration was noticed in four sheep (namely, 222, 223, 224 and 226) in the No Mo group and in one sheep each in the Low, Medium and High Mo groups, viz. 218, 241 and 230 respectively. Sheep 218 suffered from pleurisy to such an extent that half of the carcass was condemned. No evidence of the presence of liver tape worm (Stilesia hepatica) was noted, but scars, apparently due to the migratory stage of internal parasites were observed in some livers.

Serum enzymes

The GOT and LDH levels in the serum followed more or less the same pattern for each sheep throughout the trial. They are therefore presented together in Table 8.1. These enzyme levels tended to increase sharply in the case of some individual sheep as may be seen from the values presented in Appendix Tables 2 and 3. The dates on which blood was collected, have been grouped into two periods of approximately 16 weeks each and the average enzyme levels are given for these two periods (Table 8.1). Increases in GOT levels were observed in some sheep within the first six weeks, before Mo treatment commenced, and these elevated levels were usually maintained throughout the trial. In the No Mo treatment five of the eight sheep, including all four ewes, showed elevated enzyme levels towards the end of the trial. Levels of over 300 IU GOT/dm^3 were measured in four sheep, two in the No Mo treatment (223 and 224), and one each in the Low Mo and High Mo treatments (214 and 231). Very high GOT and LDH levels were measured in sheep 223. In all cases where elevated enzyme levels were observed at a reasonably early stage of the trial, there was a decreasing trend in concentration after a peak had been reached. In the case of ewes 224 and 223 the highest GOT levels were observed 3 and 11 days respectively after kidney clearance studies were done on them, though in both cases the peaks in LDH concentration was reached before this clearance study. After the second kidney clearance test on ewe 223, no change in enzyme levels was observed. None of the other ewes showed similar elevated GOT levels after kidney clearance studies had been performed on them.

The sheep which developed pleurisy (218) showed elevated GOT and LDH levels towards the end of the trial. Sheep 231 in the high Mo group showed high plasma enzyme levels throughout.

At different stages of the trial, fairly wide variations in plasma arginase levels were measured, even within sheep. This was partly due to the occurrence of hemolysis in the blood but probably also because of inaccuracies in the assay procedure. Average plasma

arginase levels are presented in Table 8.1. These tend to show trends similar to those of GOT and LDH. Substantially higher arginase levels were measured in the plasma of the No Mo group than in the other three groups. Very high average arginase levels were observed in the plasma of sheep 223, the ewe with the highest GOT and LDH levels.

Micro-anatomical evaluation

a) Liver histopathology

The results of the histopathological investigation of the livers are given in Appendix Tables 4 and 5. The degree of necrosis of isolated hepatocytes noted for each sheep is also presented in Table 8.1 for direct comparison with the serum enzyme data. The most characteristic and significant histopathological changes in the livers were necrosis of individual hepatocytes, usually with infiltration of neutrophils. Hepatocyte necrosis was present in all treatment groups but most pronounced in the group receiving high dietary levels of Cu without Mo supplementation (No Mo group). Three sheep in this group showed pronounced and three showed moderate levels of hepatocyte necrosis. Distinct cell necrosis was noted in one sheep each from the Low and the High Mo treatments(213 and 231 respectively).

Minimal to moderate enlargement of the nuclei was observed in hepatocytes, expecially in the vicinity of the portal canals. This occurred more or less equally in all treatment groups. Eosinophylic nucleus inclusions with moderate margination of chromatin were observed in sheep numbers 212, 213, 215, 235 and 237. This may indicate advanced nuclear damage.

Hydropic degeneration was observed in most sheep and was not specifically related to any treatment. Fatty changes were observed in only two sheep. Portal cell infiltration, mainly of a lymphocytic nature was observed, but this was not related to any treatment. No prominent fibrosis, gall duct proliferation nor pigmentation in the portal areas was observed in the liver of any sheep.

Table 8.1 Average serum GOT and LDH levels and plasma arginase levels in the blood of individual sheep for the periods indicated, and the histological evaluation of the livers of all sheep

	GOT IU/dm^3		LDH IU/dm^3		Arginase IU*	Liver : Hepatocyte necrosis score**
	15 Aug to 6 Dec	13 Dec to 2 April	27 Aug to 6 Dec	13 Dec to 2 April	23 Nov to 2 April	
Sheep			No Molybdenum			
219	56	70	650	789	79	1
220	70	89	653	662	147	2
221	72	115	861	1023	176	2
222	60	75	777	839	97	1
223	177	464	1292	1993	508	3
224	116	213	1115	1387	319	3
225	123	135	1019	987	374	2
226	101	126	1249	1394	217	3
Av.	97	161	952	1134	240	2.1
			Low Molybdenum			
211	55	60	549	624	51	1
212	70	65	744	873	95	2
213	69	104	791	867	108	3
214	162	183	952	985	203	2
215	68	94	734	871	156	1
216	166	122	1385	1143	241	1
217	53	54	914	768	101	1
218	73	103	901	1125	272	2
Av.	90	98	871	923	119	1,6
			Medium Molybdenum			
235	84	91	875	842	176	1
236	72	79	647	621	52	1
237	81	90	804	772	92	1
238	66	67	939	844	137	2
239	82	85	913	913	178	1
240	58	68	739	732	105	1
241	56	55	707	717	85	1
242	61	66	710	736	78	1
Av.	70	75	792	772	113	1,1
			High Molybdenum			
227	61	68	787	782	117	1
228	77	81	763	779	110	1
229	64	70	768	963	89	2
230	76	70	925	835	143	1
231	145	166	886	1153	177	3
232	70	78	783	765	94	1
233	63	68	842	881	76	1
234	72	101	738	870	173	2
Av.	75	88	812	879	122	1,5

* One IU of arginase activity is the amount of arginase required to form 1 µM urea in one hour

** Necrosis score : 1 = minimal, 2 = moderate, 3 = pronounced.

b) Kidney histopathology

No pathological lesions were apparent with Haematoxylin-Eosin staining. Distinct PAS positive granules, as small, round, red granules, were seen in the cytoplasm of the cortical tubules. In Table 8.2 the extent by which the granules were PAS positive is shown for each individual sheep. A higher frequency of intracytoplasmic granules staining PAS positive was observed in the High Mo group than in the other groups.

Globules in the interstitual tissue of the medulla stained PAS positive in most sheep, though this was not related to any treatment.

Kidney clearances

The results of the kidney clearance study are given in Appendix Table 6. A summary of these results, expressed in cm^3 plasma per kg body mass, as well as the clearances of creatinine and urea in relation to inulin, are presented in Table 8.3.

During the kidney clearance study ewe 223 in the No Mo group experienced strained breathing and started to scour while urine flow almost ceased. After three collections of the first run had been completed the test was terminated. On repeating this clearance study a few weeks later, the ewe was again under stress but this time urinated excessively. Average clearances for both runs on this sheep are presented. Values between individual collections within the second run varied widely. The results from this run (223 b) were therefore ignored in the evaluation of the data.

The data on the kidney clearances were too few to warrant definite conclusions regarding treatment effects on kidney function. Two sheep (215 and 231) showed creatinine and urea clearances in relation to inulin well above values observed for the other ewes. This was due to both a lower inulin clearance and a higher creatinine and urea clearance than in the other sheep. The ratios of creatinine

Table 8.2 The occurrence of PAS positive granules in kidney cortical tubules of sheep receiving different levels of dietary Mo

No Molybdenum		Low Molybdenum		Medium Molybdenum		High Molybdenum	
Sheep number	PAS pos.* granules	Sheep number	PAS pos.* granules	Sheep number	PAS pos.* granules	Sheep number	PAS pos.* granules
219	1	211	1	235	2	227	1
220	1	212	0	236	1	228	2
221	-	213	1	237	1	229	3
222	2	214	1	238	2	230	2
223	2	215	2	239	3	231	3
224	2	216	0	240	2	232	2
225	2	217	1	241	1	233	1
226	1	218	2	242	2	234	2
Total	11	Total	8	Total	14	Total	16

* 0 = none, 1 = minimal, 2 = moderate, 3 = pronounced

Table 8.3 Average kidney clearances of inulin, creatinine and urea of ewes per kg body mass and the ratio of creatinine and urea to inulin clearance

Treatments	Ewe Number	Clearances (/kg body mass) Inulin (C_{IN})	Creatinine (C_{CR})	Urea (C_{UR})	Ratios C_{CR}/C_{IN}	C_{UR}/C_{IN}
No Mo	223(a)	1,65	1,29	0,65	0,78	0,39
	223(b)*	2,38	2,60	1,41	1,09	0,59
	224	1,58	0,73	0,67	0,46	0,42
	225	1,72	1,10	0,77	0,64	0,45
	226	1,85	1,35	0,93	0,73	0,50
Low Mo	215	0,96	1,83	1,11	1,91	1,16
	216	1,49	1,54	1,06	1,03	0,71
	218	1,16	1,02	0,53	0,88	0,46
Med Mo	239	1,15	1,56	0,77	1,36	0,67
	241	1,28	1,41	0,70	1,10	0,55
	242	1,49	1,76	1,18	1,18	0,79
High Mo	231	0,83	3,14	2,00	3,78	2,41
	232	1,63	1,44	0,84	0,88	0,52
	233	2,08	2,21	1,39	1,06	0,67
	234	1,93	1,59	1,04	0,82	0,54

* Wide variations in clearances between collections within run, data not considered in evaluation.

to inulin clearance in the No Mo group were markedly lower than this ratio in the sheep receiving Mo. This lower ratio was mainly due to a lower creatinine clearance in this group as compared to the other groups. Although differences were not so pronounced in the C_{UR}/C_{IN} ratio of the No Mo group as in creatinine, the results for urea seemed to follow the same trend as in creatinine, viz. relatively lower urea to inulin clearances in the group that received No Mo as compared to those receiving additional Mo.

From the limited number of observations available it appears that Mo supplementation had no marked effect on kidney function in the sheep under conditions prevailing in the present experiment.

DISCUSSION

Even though the GOT and LDH levels in serum were determined with the use of standard analytical kits intended for human blood, the values obtained in the present trial corresponded well with values reported by Todd & Thompson (1963), Van Adrichem (1965), Ishmael et al.(1972) and Van der Berg & Van der Schee (1973) for normal sheep, viz. below 135 IU GOT/dm^3 and between 345 and 840 IU LDH/dm^3.

Increases in GOT and LDH levels in serum may indicate that tissue breakdown is taking place somewhere in the body (Boyd, 1962; Schmidt & Schmidt, 1967) while high serum arginase levels indicated more specifically liver tissue breakdown (Schwartz, 1971). Concentration of all three enzymes are frequently used to monitor the extent of liver tissue damage when Cu toxicosis in sheep is suspected (Todd & Thompson, 1963; Ross, 1964, 1966; Van Adrichem, 1965; Hogan et al., 1968; MacPherson & Hemingway, 1969; Ishmael et al., 1972; Van der Berg & Van der Schee, 1973). Changes in enzyme levels were detected by these authors up to eight weeks before the onset of the haemolytic crisis.

Elevated enzyme levels were measured during the present trial in sheep receiving high levels of dietary Cu without Mo and at the low level of

Mo supplementation (No Mo and Low Mo treatment groups). Possible reasons for the high serum enzyme levels in sheep 231 (High Mo group) will be discussed later. In comparing the enzyme levels with the histopathological examination of the liver, it may be concluded that these enzyme changes were the result of liver tissue breakdown, especially in the No Mo group. In the case of sheep 223, these enzyme levels were very high, but showed a decrease towards the end of the trial. Pronounced hepatocyte necrosis of the liver was also observed in this ewe. Although no haemolytic crisis was noticed in this sheep, which was being kept under close surveillance especially after the kidney clearance study, the occurrence of high serum enzyme levels suggests that this ewe may have experienced such a crisis. However, the liver and kidney lesions observed in sheep 223 were histologically different from those described by Ishmael et al. (1971 b) and Gopinath et al. (1974) as being characteristic of sheep which survived a haemolytic crisis.

The histopathological and serum enzyme levels measured in the present trial, suggests that tissue breakdown actually took place in the livers of some sheep, mainly in the group fed high Cu levels without Mo. These results substantiate those of Ishmael et al. (1971b) who observed histological changes during the pre-haemolytical stage of Cu toxicity in sheep. However, changes in enzyme concentrations were observed in the present trial for extended periods, without the occurrences of a haemolytic crisis. These results, therefore, do not support the observation by various other authors that a haemolytic crisis can be expected within eight weeks from the change in serum enzyme levels (Todd & Thompson, 1963). It is possible that the Merino types used in the present trial with their suggested higher resistance to Cu toxicosis and ability to survive more than one haemolytic crisis (Edgar et al., 1941) may also show elevated enzyme levels without advancing to the stage of the haemolytic crisis. If this is the case then elevated GOT, LDH and arginase levels in serum do not give reliable indications of an approaching haemolytic crisis in the Merino.

Inulin clearance is commonly used as a measure of the glomerular filtration rate (GFR) of the kidneys (Smith, 1956). In the case of humans and laboratory experimental animals, Smith (1956) suggested that GFR be expressed on a body surface area basis for comparative purposes. Owen (1975) expressed his results and made comparisons on a per kilogram body mass basis. Owen (1975) observed GFR values of 1,75 with a SD of $\pm$ 0,72 and 1,89 $\pm$ 0,64 cm^3/min/kg. He also quoted values from various other workers ranging from 1,75 to 2,35 cm^3/min/kg body mass. The inulin clearances observed in the present trial corresponded well with these results, except in the case of two sheep, viz. number 215 and 231, where values below 1,0 cm^3/min/kg body mass were measured.

In some species the kidney clearance of creatinine is very similar to that of inulin (Smith, 1956). However, Ladd, Liddle, Gagnon & Clarke (1957) pointed out that in sheep and goats, tubular participation in the excretion of creatinine takes place. Creatinine to inulin clearance ratios (C_{CR}/C_{IN}) of approximately 1,0 and above can be expected. Shannon (1937) measured a C_{CR}/C_{IN} of 1,03 in sheep while Ladd et al.(1957) reported ratios of between 1,1 and 1,7 in normal goats and between 0,3 and 1,4 in goats after a traumatic shock. Smith (1956) pointed out that many substances when administered simultaneously depressed tubular excretion of each other, though he emphasized that this would not indicate any toxic actions between them. Ladd et al. (1957) observed a drop in creatinine clearance with the administration of p - aminohippurate (PAH) and thiosulphate while the inulin clearance remained unaltered.

In the present trial the C_{CR}/C_{IN} ratios were within the range of 0,9 to 1,2 in all the groups receiving additional dietary Mo, except for sheep 215 and 231. The high C_{CR}/C_{IN} ratios observed in these two sheep were due, not only to a low inulin clearance, but also to a relatively high creatinine clearance, especially in the case of sheep 231. Smith (1956) pointed out that any reduction in renal filtration rate, for example, due to a renal disease, would result in a reduced amount of substances excreted by filtration such

as creatinine, uric acid and urea. The relatively high rate of creatinine and urea clearance, especially in sheep 231, is difficult to explain in view of the fact that relatively high GOT and LDH levels were measured in this sheep throughout the trial, and the concentration of PAS positive granules in the cortex tubules of this sheep were high, indicating some kidney damage.

Relatively low C_{CR}/C_{IN} ratios were observed in the ewes which did not receive any additional Mo during the experiment, as compared with those sheep receiving additional Mo. Ladd et al.(1957) reported the reduction of creatinine clearance in the presence of thiosulphate. In this present trial fairly high levels of S were fed to the sheep. Bird & Hume (1971) observed a high rate of ester sulphate, including thiosulphate, excretion through the urine when SO_4^{2-} and cystine were fed to sheep. It seems quite possible that S-containing substances in the blood reduced the creatinine excretion in the No Mo group during this trial. Gawthorne & Nader (1976) found a marked reduction in S^{2-} absorption through the rumen wall in the presence of fairly small quantities of Mo. The formation of thiomolybdates in the rumen (Dick et al., 1975; Gawthorne & Nader, 1976) may also reduce the concentration of S compounds in the blood. This may be the reason why the low rate of creatinine excretion was only observed in the treatment without Mo and not in those receiving additional Mo in the rations.

Shannon (1937) reported a C_{CR}/C_{IN} ratio of 0,52 in sheep, which corresponds well with the ratios observed in the present trial, with the exception of sheep 215 and 231.

From the kidney clearance study no impairment of kidney function, as suggested by Van Adrichem (1965), was observed at high Mo + S intakes. However, the tendency towards a higher frequency of PAS positive granules in the cortical tubules of the High Mo groups may indicate some influence of accumulated Cu and Mo in the kidneys of sheep receiving high levels of Cu, Mo and S.

CHAPTER 9

GENERAL DISCUSSION

The prevention and control of Cu and Mo toxicosis in sheep (induced hypocuprosis) is a problem of considerable practical importance. From the results of the present series of trials it is clear that the various aspects of the Cu - Mo - S interaction must be fully understood if the animal nutritionist is to be in a position to modify the Cu metabolism of his animals by dietary means. Much work in this direction has been attempted, but the reports in the literature confirm that the whole field is still in a state of disarray.

Broadly speaking, two separate aspects of Cu toxicity can be identified. On the one hand there is the undesirable accumulation of Cu in the body due to the presence of this mineral in the food consumed. On the other hand there is the question of eliminating Cu from the body in a practical manner.

The dietary approach to the problem has taken various forms. Some research workers have proposed that dietary ratios between Cu and Mo be used to identify rations likely to be satisfactory in practice. Thus, Cu : Mo ratios of 2 : 1, (Miltimore & Mason, 1971); 5 : 1 (Alloway, 1973; Pope, 1975) and 7 : 1 (Case, 1974) have all been recommended as safe, and not likely to result in Cu deficiency in sheep. Unfortunately, Cu : Mo ratios are not independent of other minerals in the ration. In the present trials, the level of dietary S was shown to exert a modifying influence such that at a low level of S (0,2%), a Cu : Mo ratio of 2 : 1 would be unlikely to result in a Cu deficiency, while at greater inclusions of S (0,4% to 0,5%), a wider ratio would be required to safeguard against hypocuprosis. The suggestion made by Suttle (1974b) that both Mo and S be taken into account when predicting the availability of Cu to sheep, therefore has considerable merit.

However, the usefulness of formulae and ratios to describe the response of Cu in the body to Cu - Mo - S interactions can have only limited scope because of the following reasons :

1. The form (organic or inorganic) or compounds in which the Cu, Mo or S are presented may influence the availability of the mineral to the body;

2. The presence of other minerals in the diet or in the body may interfere with the metabolism of Cu, Mo or S;

3. Differences in the metabolism of at least Cu, may exist between sheep breeds;

4. Decisions regarding the Cu - Mo - S ratio depend on the mineral status of the body, which is difficult to determine.

In the first place, the availability of Cu itself depends upon the chemical form in which it is consumed (Chapman & Bell, 1963). In practice, therefore, Cu toxicity in sheep may arise from the intake of different forms of Cu, the availability of which is likely to differ from source to source. Furthermore, the interaction of Cu with Mo is variable depending upon whether Mo is present in organic or in inorganic form (Suttle, 1975b; Fisher & Clawson, 1976). This latter problem may not be very serious in practice because Mo, when used prophylactically, is normally added to rations as an inorganic salt. In these circumstances its behaviour may possibly be predictable with reasonable accuracy.

Although Suttle (1974c and 1975b) proved that S in both the organic and inorganic form effects Cu metabolism with more or less equal efficiency, Goodrich & Tillman (1966) and Huisingh & Matrone (1976) observed differences in Cu metabolism depending on the form in which S was provided to sheep. Furthermore, some S may escape participation in interactions with Mo by bypassing ruminal reductions (Suttle, 1975b) and secondly, endogenous S may be added to the S pool in the rumen through the saliva (Hume & Bird, 1970). It is clear

that the contribution of S is the most unpredictable of the three minerals in the Cu - Mo - S interaction and that its interaction with Cu or with Cu + Mo cannot be ignored.

The existance of interactions between Cu and various other minerals has been demonstrated (Tillman, 1966; Hill & Matrone, 1970). However, the levels and quantities in the diet necessary to observe the presence of such interactions in sheep have, in most cases, not been determined. Bremner et al. (1976) demonstrated that Zn at levels of 220 and 420 mg/kg feed was effective in controlling hepatic Cu accumulation in sheep. From the present series of trials it has become clear that many of the minerals antagonistic to Cu in the body, may be present in rations at levels well above the requirements of sheep for those minerals. Some evidence of interactions between Cu and Fe, and Cu and Zn was observed in Trial 2. The inclusion of poultry manure in a diet should contribute substantially to the concentrations of minerals antagonistic to Cu. It is possible that increasing the amount of poultry manure in a diet may increase total Cu intake, but this will simultaneously increase the intake of Cu antagonists, rendering Cu less available to sheep.

In their work on pigs, Suttle & Mills (1966) observed an additive effect of Cu and Fe on the increase in Zn levels in pig livers. This may indicate that some of the interacting effects of two minerals on another mineral may be independent, resulting in an additive response. However, it can be expected that some of the interactions of different minerals on a given mineral will be of a non-additive nature. If, for instance S renders a proportion of Cu unavailable in the rumen by forming CuS (Huisingh et al., 1973), then less Cu will remain to interact with Zn at tissue level. The best example is probably the interactions between Cu and S, and Cu and Mo + S. Although both pathways are known to exist, it is not clear to what extent CuS or cupric thiomolybdate would be formed in the digestive tract if both Mo and S are fed at high levels.

The fact that both additive and non-additive interactions between minerals can occur, greatly diminishes the practicability of

developing multiple regression equations for predicting the relationships between dietary minerals and Cu metabolism. It would seem that a solution to this problem must be sought by other approaches than simple mathematical formulae. Various considerations tend to support this conclusion. Under experimental conditions it is practically impossible to look at all possible interactions of the minerals under investigation. With the use of purified diets, the researcher can ensure the addition of nutrients in the "right" quantities and may be able to develop prediction equations. With natural feeds, however, with variable and often even unknown amounts of a large array of minerals present, the position becomes unmanageable. In the present series of trials a knowledge of the Cd and Se content of the diets could possibly also have been useful, especially where poultry manure was included.

Aside from the mineral content of rations, information about other minerals and mineral levels in the blood may also be useful background information against which results can be evaluated, eg. although Co does not apparently interact with Cu, anaemia is a deficiency symptom of both Co and Cu (Ammerman, 1970). A knowledge of the Co intake might have helped in interpreting results such as the anaemia observed in sheep during Trial 2 of this series.

Genetic differences in ability to accumulate Cu in the liver or to survive a haemolytic crisis have been observed between sheep breeds (Edgar et al., 1941; Marston & Lee, 1948). The extent of these differences has not been quantified for breeds. Within breeds, variation also seems to be wide (Todd et al., 1962). If breed differences are large any formula or ratio to predict the response of the Cu - Mo - S interaction on Cu accumulation in the liver, will have to be breed specific. In the present series of trials, it is possible that a breed effect was responsible for the low hepatic Cu retentions observed. Resistance to Cu toxicity was also suggested as an explanation for the prolonged occurrence of elevated serum GOT levels in the Merino type of sheep on high levels of dietary Cu during Trial 4.

In view of foregoing considerations, the problem of a suitable experimental design for studies of mineral nutrition, eg. Cu, is a matter of some importance. Sheep can be blocked according to liver Cu concentration at the onset of a trial to ensure equal average Cu concentrations per treatment. This can be achieved by the use of liver biopsy technique, as suggested by Dick (1954), and would probably reduce some of the variation in liver Cu levels at the end of a trial. If the wide variation in rate of accumulation of dietary Cu in the liver within a breed has a genetic basis, some breeds may be preferable to others as experimental subjects.

As an aside, genetic differences in resistance to Cu toxicity create the possibility of selection for this trait. For success, a rapid and reliable method for assessing resistance would be required. Liver biopsy is not a very attractive technique, but if the ability of sheep to metabolise Cu can be measured by evaluating the turn-over rate of plasma caeruloplasmin, progress could be possible. For the present, caeruloplasmin turnover rate is evaluated using expensive Cu^{67}, making this approach rather impracticable (Marcilese et al., 1976).

A knowledge of the Cu intake of sheep will be of valuable assistance when deciding on amounts of Mo and S to be supplied in relation to dietary Cu. As long as definite relationships between dietary Cu, Mo and S on Cu metabolism have not been established, the liver Cu content should be monitored to ensure that a state of Cu deficiency does not develop.

When the Cu content of the liver has to be reduced, Mo and S should be supplied at levels high enough to promote the systemic interactions between Cu, Mo and S. However, it is not possible to suggest specific levels of Mo and S for general use. From the results of the present trials, and from results in the literature, it is apparent that Mo and S levels at which these systemic effects can be expected, differ quite widely (Smith et al., 1968; Marcilese et al., 1970; Bingley, 1974 and Smith & Wright, 1975b), probably because of

all the factors which can influence the Cu, Mo and S metabolism in the body. Again, the Cu status of the liver will have to be followed to decide when to stop treatment and so to avoid hypocuprosis. The systemic effect may result in a build-up of Cu and Mo in the liver in forms unavailable to the body. This decreases the accuracy of evaluating the Cu status of the body from the hepatic Cu concentration. No information is available on the level of Cu, Mo or S intake at which the unavailable Cu-Mo compound will start to accumulate in the liver.

Liver Cu concentrations can be estimated reasonably precisely from the Cu content of liver biopsy samples, despite variations in Cu concentration at different sites of the liver (Barden & Robertson, 1962). Tissue and plasma concentrations of Mo tend to fluctuate according to Mo intake (Cunningham & Hogan, 1959; Lesperance & Bohman, 1963). Relative values depend on S intake (Trial 3) and give no indication of the Mo status of the animal or the extent of the participation of Mo in the Cu - Mo - S interaction. In fact, the present series of trials indicated that Mo levels in the body fluctuate according to dietary Mo concentration excluding any indication of the long term Mo status of the body. The build-up of Mo in bone was not investigated in these trials, and may be related to a long-term accumulation of Mo in the body (Cunningham & Hogan, 1959; Lesperance & Bohman, 1963). The presence of Mo in unavailable combinations with Cu in body tissue, due to the systemic effect of the Cu - Mo - S interaction may, however, be of a more long-term nature than the available Mo. The Mo bound in this way may be of no value in evaluating the Mo status of the animal.

Under practical conditions the use of the liver biopsy technique to determine the Cu status of the animal is hardly practicable. Total Cu intake of a grazing animal will also be difficult to determine. The stockman and his advisers will, therefore, have to rely on formulae for predicting the Cu metabolism and the effects of the Cu - Mo - S interaction on it. Information about deaths of contemporaries and a knowledge of the Cu toxicity problem in the

area will help in decision-making. Such a hit-and-miss method is unsatisfactory and very big margins of safety will have to be allowed for.

It is obvious that safer and more reliable methods of controlling Cu toxicity should be sought. The use of high levels of Zn as an antagonist to Cu has been suggested (Bremner & Davies, 1973: Mills, 1974) and tested (Bremner et al., 1976). The interaction between Cu and Zn was suggested to take place at tissue level (Hill & Matrone, 1970), including the liver (Bremner & Marshall, 1974).

The interaction between S and Cu with the formation of CuS has been shown to take place mainly in the digestive tract of the ruminant (Huisingh et al., 1973; Suttle, 1974b). This interaction may be exploited to control Cu absorption in sheep. From available evidence it is not clear to what extent S would prevent Cu absorption, though this will depend on the level of S supplementation in relation to dietary Cu levels.

Interactions with other minerals in the diet will have to be taken into account when S or Zn are used to control Cu toxicosis in sheep. S was found to interfere also with the metabolism of Se, Ca and P in the body (Goodrich & Tillman, 1966; Tillman, 1966) while Zn can also interact with Fe, S and P (Tillman, 1966). These interactions may reduce the value of using these two minerals and may result in unreliable predictions of changes in Cu metabolism in the body.

Thiomolybdates or ammonium thiomolybdates were suggested as possible compounds responsible for the binding of Cu, both in the digestive tract and in the body (Dick et al., 1975; Fisher & Clawson, 1976). The intravenous injection of thiomolybdate in known quantities to bind and inactivate a known concentration of Cu in the body shows promise of a means of preventing Cu toxicosis in sheep on a controlled basis. Furthermore, through oral administration of thiomolybdates, Cu absorption from the digestive tract can be reduced, provided

thiomolybdates are not degraded in the rumen. However, the role of thiomolybdates in the Cu - Mo - S interaction will have to be elucidated and dose-response effects must be determined. Whether thiomolybdates are the only compounds responsible for the Cu - Mo - S interaction, is not clear. It has been shown by Gawthorne & Nader (1976) that thiomolybdates are formed in the rumen. The accumulation of Cu and Mo in fixed ratios in rats' livers has been observed by Mills & Mitchell (1971). This suggests the formation of Cu and Mo containing compounds in the body of the non-ruminant.

It is clear that many aspects of the Cu - Mo - S interaction in sheep have still to be clarified. Predictions of changes in Cu metabolism in the body, based on Cu, Mo and S intakes, will be very specific to the situation under which they were calculated, i.e. type of feed, feed ingredients and breed of sheep. It is clear from the present series of trials that the most informative results will be obtained if interacting effects between these minerals are investigated at more than two levels of a specific mineral. However, this requires a factorial type of experimental design involving many animals and treatments.

REFERENCES

ABDELLATIF, A.M.M., 1968. Conditioned hypocuprosis : some effects of diet on copper storage in ruminants. Verslagen van Landbouwkundige Onderzoekingen, No 709. Wageningen.

ADAMSON, A.H., VALKS, D.A., APPLETON, M.A. & SHAW, W.B., 1969. Copper toxicity in housed lambs. Vet. Rec. 85, 368.

ALLOWAY, B.J., 1973. Copper and molybdenum in swayback pastures. J. agric. Sci., Camb. 80, 521.

AMMERMAN, C.B., 1970. Recent developments in cobalt and copper in ruminant nutrition : A review. J. Dairy Sci. 53, 1097.

ANONYMOUS, 1960. Dorp het plaas geword. Landbouweekblad. Des.6,66.

A O A C. , 1965. Official methods of analysis of the Association of Official Agricultural Chemists. 10th ed. Washington, D.C. : Benjamin Franklin Sta.

ARTHUR, D., 1965. Interrelationships of molybdenum and copper in the diet of the guinea pig. J. Nutr. 87, 69.

AWAD, Y.L., AHMED, A.A., LOTFI, A.Y. & FAHMY, F., 1973. The influence of selenium administration on copper levels and growth of lambs. Zentralb. Veterinarmed. 20, 742.

BATEY, T., BERRYMAN, C. & LINE, C., 1972. The disposal of copper-enriched pig-manure slurry on grassland. J. Br. Grassl. Soc. 27, 139.

BATH, G.F., 1979. Enzootic icterus - a form of chronic copper poisoning. J. S. Afr. vet. Assoc. 50, 3.

BARDEN, P.J. & ROBERTSON, A., 1962. Experimental copper poisoning in sheep. Vet. Rec. 74, 252.

BECK, A.B., 1963. The copper metabolism of warm-blooded animals with special reference to the rabbit and the sheep. Aust.J.agric. Res. 14, 129.

BINGLEY, J.B., 1974. Effects of high doses of molybdenum and sulphate on the distribution of copper in plasma and in blood of sheep. Aust. J. agric. Res. 25, 467.

BIRD, P.R. & HUME, I.D., 1971. Sulphur metabolism and excretion studies in ruminants. IV. Cystine and sulphate effects upon the flow of sulphur from the rumen and upon sulphur excretion by sheep. Aust. J. agric. Res. 22, 443.

BISHOP, E.J.B., WILKE, P.I., NASH, W.J., NELL, J.A.G., MACDONALD, D.A., COMPAAN, J.P., GROBLER, J. & KINGMAN, E.R., 1971. Poultry manure as a livestock feed (Part 1). Fmg. S. Afr. 46, (11), 34.

BLAIR, R., 1974. Utilization of wastes and by-products in animal feeds. Feedstuffs. 46, (39), 19.

BLAIR, R., 1975. Utilizing wastes in animal feeds - A European overview. Feedstuffs. 47, (26), 14.

BLAMEY, F.P.C., 1971. Molybdenum in maize (Zea Mays L.) production in Northern Natal. M. Sc. Agric. Thesis, University of Natal.

BLANCHAR, R.W., REHM, J. & CALDWELL, A.C., 1965. Sulphur in plant material by digestion with nitric and perchloric acid. Proc. Soil Sci. Soc. Am. 29, 71.

BOYAZOGLU, P.A., BARRETT, E.L. & DU TOIT, J.De V., 1972. Sulphur containing licks and their effect on the mineral-vitamin balance of sheep. S. Afr. J. Anim. Sci. 2, 65.

BOYD, J.W., 1962. The comparative activity of some enzymes in sheep, cattle and rats - normal serum and tissue levels and changes during experimental liver necrosis. Res. vet. Sci. 3, 256.

BRACEWELL, C.D., 1958. A note on jaundice in housed sheep. Vet. Rec. 70, 342.

BRAY, A.C., 1969. Sulphur metabolism in sheep. II. The absorption of inorganic sulphate and inorganic sulphate from the sheep's rumen. Aust. J. agric. Res. 20, 739.

BREMNER, I., 1976. The effect of dietary molybdenum and sulphate on plasma copper distribution in sheep. Proc. Nutr. Soc. 35, 21A.

BREMNER, I. & DALGARNO, A.C., 1973. Iron metabolism in the veal calf. The availability of different iron compounds. Br. J. Nutr. 29, 229.

BREMNER, I. & DAVIES, N.T., 1973. Trace metal interactions in animal nutrition. Rowett Res. Inst. Annual Report.29, 126.

BREMNER, I. & MARSHALL, R.B., 1974. Hepatic copper- and zinc-binding proteins in ruminants. Br. J. Nutr. 32, 283.

BREMNER, I. & YOUNG, B.W., 1978. Effects of dietary molybdenum and sulphur on the distribution of copper in plasma and kidneys of sheep. Br. J. Nutr. 39, 325.

BREMNER, I., YOUNG, B.W. & MILLS, C.F., 1976. Protective effect of zinc supplementation against copper toxicosis in sheep. Br. J. Nutr. 36, 551.

BROWN, J.M.M., 1968. Biochemical studies on geeldikkop and enzootic icterus. Onderstepoort J. vet. Res. 35, 319.

BRYDEN, M. McG. & BRAY, A.C., 1972. The effect of dietary molybdenum on the reduction of inorganic sulphate in the rumen. Proc. Aust. Soc. Anim. Prod. 9, 335.

CASE, A.A., 1974. Toxicity of various chemical agents to sheep. J. Am. vet. med. Assoc. 164, 277.

CHAPMAN, H.L. & BELL, M.C., 1963. Relative absorption and excretion by beef cattle of copper from various sources. J. Anim. Sci. 22, 82.

CLAYPOOL, D.W., ADAMS, F.W., PENDELL, H.W., HARTMANN, Jr. N.A. & BONE, J.F., 1975. Relationship between the level of copper in the blood plasma and liver of cattle. J. Anim. Sci. 41, 911.

CLEGG, F.G., 1956. An outbreak of copper poisoning in sheep. Vet. Rec. 68, 332.

COMBS, G.E., AMMERMAN, C.B., SHIRLEY, R.C. & WALLACE, H.D., 1966. Effect of source and level of dietary protein on pigs fed high-copper rations. J. Anim. Sci. 25, 613.

COOK, G.A., LESPERANCE, A.L., BOHMAN, V.R. & JENSEN, E.H., 1966. Interrelationship of molybdenum and certain factors to the development of the molybdenum toxicity syndrome. J. Anim. Sci. 25, 96.

CORBETT, W.S., SAYLOR, W.W., LONG, T.A. & LEACH, R.M., 1978. Intracellular distribution of the hepatic copper in normal and copper-loaded sheep. J. Anim. Sci. 47, 1174.

CORNELIUS, C.E. & KARNEKO, J.J., 1963. Clinical Biochemistry of Domestic Animals. New York : Academic Press.

CUNNINGHAM, I.J. & HOGAN, K.G., 1959. High molybdenum intake and the thrift of young sheep. N.Z.J. agric. Res. 2, 134.

CUNNINGHAM, I.J., HOGAN, K.G. & LAWSON, B.M., 1959. The effect of sulphate and molybdenum on copper metabolism in cattle. N.Z.J. agric. Res. 2, 145.

DALE, S.E., EWAN, R.C., SPEER, V.C. & ZIMMERMAN, D.R., 1973. Copper, molybdenum and sulfate interaction in young swine. J. Anim. Sci. 37, 913.

DALGARNO, A.C. & MILLS, C.F., 1975. Retention by sheep of copper from aerobic digests of pig faecal slurry. J. agric. Sci., Camb. 85, 11.

DAVIES, N.T., 1974. Recent studies of antagonistic interactions in the aetiology of trace element deficiency and excess. Proc. Nutr. Soc. 33, 293.

DE KOCK, G., 1928. A study of the reticulo-endothelial system of the sheep. 13th & 14th Reports of the Div. of vet. Educ. & Res. 647.

DENNY, J., 1977. Dried poultry waste today. S A P A Poultry Bull. June. 183.

DICK, A.T., 1954. Studies on the assimilation and storage of copper in crossbred sheep. Aust. J. agric.Res. 5, 511.

DICK, A.T., 1956. Molybdenum and copper relationships in animal nutrition. In : Inorganic Nitrogen Metabolism.pg. 445. Ed. McElroy, W.D. & Glass, B. Baltimore : John Hopkins Press.

DICK, A.T., DEWEY, D.W. & GAWTHORNE, J.M., 1975. Thiomolybdates and the copper-molybdenum-sulphur interaction in ruminant nutrition. J. agric. Sci., Camb. 85, 567.

DIRKSEN, G. & HOFMANN, W., 1974. Actual health problems in the rearing and fattening of calves - A review. Vet. med. Rev. 1, 3.

DOWDY, R.P. & MATRONE, G., 1968. A copper - molybdenum complex : Its effects and movement in the piglet and sheep. J. Nutr. 95, 197.

EDEN, A., 1940. Observations on copper poisoning. J. comp. Path. Therap. 53, 90.

EDGAR, G., HINDMARSH, W.L., KEAST, J.C. & ROSE, A.L., 1941. The distribution and incidence of enzootic or toxaemic jaundice in South-Eastern Australia with special reference to New South Wales. Aust. vet. J. 17, 120.

EMERSON, J., 1975. Feed prices, environmental laws help sales outlook for drying equipment, DPW. Feedstuffs. 47, (3), 32.

ERASMUS, J.A., 1970. Copper metabolism in the Merino sheep in South Africa. Normal levels of liver copper in sheep from the Cape Midlands. J. S. Afr. vet. med. Assoc. 41, 207.

ESSIG, H.W., 1975. Recycling nutrients for livestock. Feedstuffs 47, (21),35.

FIELD, A.C., MUNRO, C.S. & SUTTLE, N.F., 1977. Dried poultry manure as a source of phosphorus for sheep. J. agric. Sci., Camb. 89, 599.

FISHER, G.L. & CLAWSON, W.J., 1976. Western United States field trials and radioisotopic investigations on the molybdenum-copper interactions in livestock. IN : Nuclear Techniques in Animal Production and Health. pg. 97. I A E A & F A C, Vienna.

FISHER, C., LAURSEN-JONES, A.P., HILL, K.J. & HARDY, W.S., 1973. The effect of copper sulphate on performance and the structure of the gizzard in broilers. Br. Poult. Sci. 14, 55.

FISHER, C., WISE, D. & FILMER, D.G., 1972. The effect of copper on the growth of broilers and the interaction of copper with zinc and iron. 14th Wld. Poult. Congr; Madrid. 2, 759.

FONTENOT, J.P., WEBB, K.E., LIBKE, K.G. & BUEHLER, R.J., 1971. Performance and health of ewes fed broiler litter. J. Anim. Sci. 33, 283. (Abstr.)

FOX, M.R.S. & REYNOLDS, H., 1973. Trace minerals in food : Nutritional - toxicological problems. Feedstuffs. 45, (13), 30.

FROST, D.V., 1979. Essential but toxic trace elements : A changing picture. Feedstuffs. 51, (37), 25.

GARNER, R.J., 1963. Veterinary Toxicology. 2nd ed. London : Tindall & Cox.

GAWTHORNE, J.M. & NADER, C.J., 1976. The effect of molybdenum on the conversion of sulphate to sulphide and microbial-protein- sulphur in the rumen of sheep. Br. J. Nutr. 35, 11.

GIPP, W.F., POND, W.G., KALLFELZ, F.A., TASKER, J.B., VAN CAMPEN, D.R., KROOK, L. & VISEK, W.J., 1974. Effect of dietary copper, iron and ascorbic acid levels on hematology, blood and tissue copper, iron and zinc concentrations and Cu^{64} and Fe^{59} metabolism in young pigs. J. Nutr. 104, 532.

GOODRICH, R.D. & TILLMAN, A.D., 1965. Effects of sulphur and nitrogen sources and copper levels in the metabolism of certain minerals by sheep. J. Anim. Sci. 25, 484.

GOPINATH, C., HALL, G.A. & HOWELL, J. McC., 1974. The effect of chronic copper poisoning on the kidneys of sheep. Res. vet. Sci. 16, 57.

GRACE, N.D. & SUTTLE, N.F., 1979. Some effects of sulphur intake on molybdenum metabolism in sheep. Br. J. Nutr. 41, 125.

GRACEY, H.I., STEWARD, T.A., WOODSIDE, J.D. & THOMPSON, R.H., 1976 The effect of disposing high rates of copper-rich pig slurry on grassland on the health of grazing sheep. J. agric. Sci., Camb. 87, 617.

GRACEY, J.F. & TODD, J.R., 1960. Chronic copper poisoning in sheep following the use of copper sulphate as a molluscicide. Br. vet. J., 116, 405.

HARKER, D.B., 1976. The use of molybdenum for the prevention of nutritional copper poisoning in housed sheep. Vet. Rec. 99, 78.

HEDGES, J.D. & KORNEGAY, E.T., 1973. Interrelationship of dietary copper and iron as measured by blood parameters, tissue stores and feedlot performance of swine. J. Anim. Sci. 37, 1147.

HEMINGWAY, R.G. & MACPHERSON, A., 1967. The accumulation of copper in the liver of housed blackface lambs. Vet. Rec. 81, 695.

HILL, C.H. & MATRONE, G., 1970. Chemical parameters in the study of *in vivo* and *in vitro* interactions of transition elements. Fed. Am. Soc. exp. Biol. 29, 1474.

HILL, R. & WILLIAMS, H.L., 1965. The effect on intensively reared lambs of diets containing excess copper. Vet. Rec. 77, 1043.

HOGAN, K.G., MONEY, D.F.L. & BLAYNEY, A., 1968. The effect of a molybdate & sulphate supplement on the accumulation of copper in the livers of penned sheep. N.Z. J. agric. Res. 11, 435.

HOGAN, K.G., MONEY, D.F.L. & WALKER, R.S., 1971. The distribution of copper in the liver of pigs and sheep and its effect on the value of chemical analyses made on biopsy samples. N.Z.J. agric. Res. 14, 132.

HOGAN, K.G., RIS, D.R. & HUTCHINSON, A.J., 1966. An attempt to produce copper deficiency in sheep by dosing molybdate and sulphate. N.Z. J. agric. Res. 9, 691.

HUBER, J.T., PRICE, W.D. & ENGEL, R.W., 1971. Response of lactating dairy cows to high levels of dietary molybdenum. J. Anim. Sci. 32, 364.

HUISINGH, J., GOMEZ, G.G. & MATRONE, G., 1973. Interaction of copper, molybdenum & sulphate in ruminant nutrition. Fed. Am. Soc. exp. Biol. 32, 1921.

HUISINGH, J. & MATRONE, G., 1976. Interactions of molybdenum in animal nutrition. In : Molybdenum in the Environment. Vol. I, pg. 125. Ed. Chappell, W.R. & Petersen, K.K. New York : Marcel Dekker, INC.

HUMASON, G.L., 1962. Animal Tissue Techniques. London : W.H. Freeman & Co.

HUME, I.D. & BIRD, P.R., 1970. Synthesis of microbial protein in the rumen. IV. The influence of the level and form of dietary sulphur. Aust. J. agric. Res. 21, 315.

ISHMAEL, J., GOPINATH, C. & HOWELL, J.McC., 1971a. Studies with copper calcium E.D.T.A. Acute toxicity in housed sheep. J. comp. Path. 81, 279.

ISHMAEL, J., GOPINATH, C. & HOWELL, J.McC., 1971b. Experimental chronic copper toxicity in sheep. Histological and histochemical changes during the development of the lesions in the liver. Res. vet. Sci. 12, 358.

ISHMAEL, J., GOPINATH, C. & HOWELL, J.McC., 1972. Experimental chronic copper toxicity in sheep. Biochemical and haematological studies during the development of lesions in the liver. Res. vet. Sci. 13, 22.

JIMENEZ, A.A., 1974. Feeding pelleted dried poultry litter to Holstein steers. Feedstuffs. 46, (47), 29.

KIRCHCESSNER, M. & GRASSMANN, E., 1970. The dynamics of copper absorption. In: Trace Element Metabolism in Animals. pg. 277. Ed. Mills, C.F. Edinburgh: E & S Livingstone.

KNEALE, W.A. & SMITH, P., 1977. The effects of applying pig slurry containing high levels of copper to sheep pastures. Expl. Husb. 32, 1.

KOOPMAN, J.J., 1963. Chronische kopervergiftiging bij schapen en lammeren. Tijdschr. Diergeneesk. 88, 1308.

KOWALCZYK, T., POPE, A.L., BERGER, K.C. & MUGGENBURG, B.A., 1964. Chronic copper toxicosis in sheep fed dry feed. J. Am. vet. med. Assoc. 145, 352.

KOWALCZYK, T., POPE, A.L. & SORENSEN, D.K., 1962. Chronic copper poisoning in sheep resulting from free-choice, trace-mineral-salt ingestion. J. Am. vet. med. Assoc. 141, 362.

LADD, M., LIDDLE, L., GAGNON, J.A. & CLARKE, R.W., 1957. Glomerules and tubular functions in sheep and goats. J. Appl. Phys. 10, 249.

LAFENETRE, H., MONTEIL, L. & GALTIER, F., 1935. Connection between haemorrhagic jaundice of sheep and copper poisoning. Rev. vet. Toulouse. 87, 263. (Abs.) Vet. Bull. 5, 1935, 855.

LAWLOR, M.J., SMITH, W.H. & BEESON, W.M., 1965. Iron requirements of the growing lamb. J. Anim. Sci. 24, 742.

LESPERANCE, A.L. & BOHMAN, V.R., 1963. Effect of inorganic molybdenum and type of roughage on the bovine. J. Anim. Sci. 22, 686.

LOOSMORE, R.M., 1969. Hazards to health. Vet. Rec. 84, 280.

LOWMAN, B.G. & KNIGHT, D.W., 1970. A note on the apparent digestibility of energy and protein in dried poultry excreta. Anim. Prod. 12, 525.

LUCKEY, T.D. & VENUGOPAL, T.D., 1977. Mineral Toxicity in Mammals. Vol. 1. New York : Plenum Press.

MACPHERSON, A., BROWN, N.A. & HEMINGWAY, R.G., 1964. The relationship between the concentration of copper in the blood and livers of sheep. Vet. Rec. 76, 643.

MACPHERSON, A. & HEMINGWAY, R.G., 1965. Effects of protein intake on the storage of copper in the liver of sheep. J. Sci. Fd. agric. 16, 220.

MACPHERSON, A. & HEMINGWAY, R.G., 1969. The relative merit of various blood analyses and liver function tests in giving an early diagnosis of chronic copper poisoning in sheep. Br. vet. J. 125, 213.

MARCILESE, N.A., AMMERMAN, C.B. VALSECCHI, R.M. DUNAVANT, B.G. & DAVIS, G.K., 1969. Effect of dietary molybdenum and sulphate upon copper metabolism in sheep. J. Nutr. 99, 177 .

MARCILESE, N.A., AMMERMAN, C.B., VALSECCHI, R.H., DUNAVANT, B.G. & DAVIS, G.K., 1970. Effect of dietary molybdenum and sulphate upon urinary excretion of copper in sheep. J. Nutr. 100, 1399.

MARCILESE, N.A., FIGUEIRAS, H.D. & VALSECCHI, R.M., 1976. Studies with copper-67 and copper-64 in conditioned copper-deficient ruminants: Preliminary results. In: Nuclear Techniques in Animal Production and Health. pg. 117. IAEA & FAO, Vienna.

MARSTON, H.R. & LEE, H.J., 1948. The effect of copper deficiency and of chronic overdosage with copper on Border-Leicester and Merino sheep. J. agric. Sci., Camb. 38, 229.

MASON, J. & CARDIN, C.J., 1977. The competition of molybdate and sulphate ions for a transport system in the ovine small intestine. Res. vet. Sci. 22, 313.

McDONALD, I.W., 1970. Mineral status of grazing ruminants in Australia. In: Australian Grasslands. pg. 371. Ed. Moore, R.M. Canberra: Australian National University Press.

MERTZ, W., 1976. Trace elements in animal nutrition. In: Nuclear Techniques in Animal Production and Health. pg. 3. IAEA & FAO. Vienna.

MILLER, R.F., PRICE, N.O. & ENGEL, R.W., 1956. Added dietary inorganic sulfate and its effect upon rats fed molybdenum. J. Nutr. 60, 539.

MILLS, C.F., 1954. Copper complexes in grassland herbage. Biochem. J. 57, 603.

MILLS, C.F., 1960. Comparative studies of copper, molybdenum and sulphur metabolism in the ruminant and the rat. Proc. Nutr. Soc. 19, 162.

MILLS, C.F., 1974. Trace-element interactions: Effects of dietary composition on the development of imbalance and toxicity. In: Trace Element Metabolism in Animals - 2. pg.79. Ed. Hoekstra, W.G., Suttie, J.W., Ganther, H.E. & MERTZ, W. Baltimore: University Park Press.

MILLS, C.F. & MITCHELL, R.L., 1971. Copper and molybdenum in subcellular fractions of rat liver. Br. J. Nutr. 26, 117.

MILNE, D.B. & WESWIG, P.H., 1968. Effect of supplementary copper on blood and liver copper-containing fractions in rats. J. Nutr. 95, 429.

MILTIMORE, J.E. & MASON, J.L., 1971. Copper to molybdenum ratio and molybdenum and copper concentrations in ruminant feeds. Can. J. Anim. Sci. 51, 193.

MURTY, V.N., 1957. The minimum copper requirement of sheep. J Scientific & Ind. Res. 16, 121.

NEATHERY, M.W. & MILLER, W.J., 1977. Tolerance levels, toxicity of essential trace elements for livestock and poultry. Feedstuffs. 49, (35), 18.

NEETHLING, L.P., BROWN, J.M.M. & DE WET, P.J., 1965. The low toxicity absorption, turnover and excretion of copper in the Merino sheep. J. S. Afr. vet. med. Assoc. 39, 13.

N R C., 1975. Nutrient requirements of domestic animals. Nutrient requirements of sheep. National Academy of Sciences, Washington, D.C.

O'DONOVAN, P.B., SPILLANE, T.A. & O'GRADY, J.G., 1966. A note on copper retention by pigs fed on various protein sources. Anim. Prod. 8, 333.

OGILVIE, D.D., 1954. "Chronic" copper poisoning of sheep. Vet. Rec. 66, 297.

OWEN, N.C., 1975. Some physiological aspects of renal function in in the sheep. D.V. Sc. thesis, University of Pretoria.

PEARCE, G.R., 1975. The inclusion of pig manure in ruminant diets. In : Managing Livestock Wastes. Proc. 3rd int. Symp. on Livestock wastes. A S A E . Publ. pg. 275. St. Joseph, Michigan.

PEARSON, J.K.L., 1956. Copper poisoning in sheep following the feeding of a copper-supplemented diet. Vet. Rec. 68, 766.

PIERSON, R.E. & AANES, W.A., 1958. Treatment of chronic copper poisoning in sheep. J. Am. vet. med. Assoc. 133, 307.

POPE, A.L., 1975. Mineral interrelationships in ovine nutrition. J. Am. vet. med. Assoc. 166, 264.

PRICE, J. & SUTTLE, N.F., 1975. The availability to sheep of copper in pig-slurry and slurry-dressed herbage. Proc. Nutr. Soc. 34, 9A.

RAYNER, A.A., 1967. Biometry for Agriculture Students. Pietermaritzburg : University of Natal Press.

ROSS, D.B., 1964. Chronic copper poisoning in lambs. Vet. Rec. 76, 875.

ROSS, D.B., 1966. The diagnosis, prevention and treatment of chronic copper poisoning in housed lambs. Br. vet. J. 122, 279.

ROSS, D.B., 1970. The effect of oral ammonium molybdate and sodium sulphate given to lambs with high liver copper concentrations. Res. vet. Sci. 11, 295.

RUSSELL, F.C. & DUNCAN, D.L., 1956. Minerals in pasture : Deficiencies and excesses in relation to animal health. 2nd ed. Commonwealth Bureau of Animal Nutrition. Tech. Comm. No 15. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks.

SCAIFE, J.F., 1956. Molybdenum excretion and retention in the sheep. N.Z.J. Sci. Tech . 38, 293.

SCHMIDT, E. & SCHMIDT, F.W., 1967. Guide to Practical Enzyme Diagnosis. Mannheim : C.F. Boehringer & Soehne GmbH.

SCHWARTZ, M.K., 1971. Clinical aspects of arginase. In : Methods in Enzymology, Vol. 17B,pg.857. Ed. Colowick, S.P. & Kaplan, N.O. New York : Academic Press.

SHAND, A. & LEWIS, G.,1957. Chronic copper poisoning in young calves. Vet. Rec. 69, 618.

SHANNON, J.A., 1937. Excretion of inulin, creatinine, xylose and urea in the sheep. Proc. Soc. Exp. Biol. & Med. 37, 379.

SMITH, B.S.W., FIELD, A.C. & SUTTLE, N.F., 1968. Effect of intake of copper, molybdenum and sulphate on copper metabolism in the sheep. III. Studies with radio-active copper in male castrated sheep. J. comp. Path. 78, 449.

SMITH, B.S.W. & WRIGHT, H., 1975a. Copper : Molybdenum interaction. Effect of dietary molybdenum on the binding of copper to plasma proteins in sheep. J. comp. Path. 85, 299.

SMITH, B.S.W. & WRIGHT, H., 1975b. Effect of dietary Mo on Cu metabolism. Evidence for the involvement of Mo in abnormal binding of Cu to plasma protein. Clin. Chim. Acta. 62, 55.

SMITH, B.S.W. & WRIGHT, H., 1976. Effects of dietary molybdenum on the metabolism in sheep of copper - 64 injected intravenously in different chemical forms. In : Nuclear Techniques in Animal Production and Health. pg. 109. IAEA & FAO, Vienna.

SMITH, H.W., 1956. Principles of Renal Physiology. New York : Oxford University Press.

SMITH, L.W., 1973. Recycling animal wastes as protein sources. In : Alternative Sources of Protein for Animal Production. pg. 146. National Academy of Science, Washington, D.C.

SMITH, M.S., 1969. Responses of chicks to dietary supplements of copper sulphate. Br. Poult. Sci. 10, 97.

SNEDECOR, G.W., 1959. Statistical Methods. 5th ed. Ames, Iowa : Iowa State College Press.

STANDISH, J.F., AMMERMAN, C.B., SIMPSON, C.F., NEAL, F.C. & PALMER, A.Z., 1969. Influence of graded levels of dietary iron, as ferrous sulphate on performance and tissue mineral composition of steers. J. Anim. Sci. 29, 496.

SUTTLE, N.F., 1974a. A technique for measuring the biological availability of copper to sheep, using hypocupraemic ewes. Br. J. Nutr. 32, 395.

SUTTLE, N.F., 1974b. Recent studies of the copper-molybdenum antagonism. Proc. Nutr. Soc. 33, 299.

SUTTLE, N.F., 1974c. Effects of organic and inorganic sulphur on the availability of diet copper to sheep. Br. J. Nutr. 32, 559.

SUTTLE, N.F., 1975a. Changes in the availability of dietary copper to young lambs associated with age and weaning. J. agric. Sci., Camb. 84, 255.

SUTTLE, N.F., 1975b. The role of organic sulphur in the copper-molybdenum-S interrelationship in ruminant nutrition. Br. J. Nutr. 34, 411.

SUTTLE, N.F. & FIELD, A.C., 1968. Effect of intake of copper, molybdenum and sulphate on copper metabolism in sheep. I. Clinical condition and distribution of copper in blood of the pregnant ewe. J. comp. Path. 78, 351.

SUTTLE, N.F. & FIELD, A.C., 1970. Effect of dietary calcium and phosphorus concentrations on the faecal excretion of copper, manganese and zinc in sheep. Proc. Nutr. Soc. 29, 33A.

SUTTLE, N.F. & FIELD, A.C., 1974. The effect of dietary molybdenum on hypocupraemic ewes treated by subcutaneous copper. Vet. Rec. 95, 166.

SUTTLE, N.F., FIELD, A.C. & BARLOW, R.M., 1970. Experimental copper deficiency in sheep. J. comp. Path. 80, 151.

SUTTLE, N.F. & MILLS, C.F., 1966. Studies of the toxicity of copper to pigs. 1. Effects of oral supplements of zinc and iron salts on the development of copper toxicosis. Br. J. Nutr. 20, 135.

SUTTLE, N.F., MUNRO, C.S. & FIELD, A.C., 1978. The accumulation of copper in the liver of lambs on diets containing dried poultry waste. Anim. Prod. 26, 39.

SUTTLE, N.F. & PRICE, J., 1976. The potential toxicity of copper-rich animal excreta to sheep. Anim. Prod. 23, 233.

TAUSSKY, H.H. & SHORR, E., 1953. A micro-colorimetric method for the determination of inorganic phosphorus. J. Biol. Chem. 202, 675.

THEILER, A., 1912. Experiments to determine the safe dose of white arsenic, Cooper's Dip and Bluestone for sheep. Agric. J. Union of S. Afr. 3, 321.

THORNTON, I., 1974. Biogeochemical and soil ingestion studies in relation to the trace-element nutrition of livestock. In: Trace Element Metabolism in Animals - 2. p. 451. Ed. Hoekstra, W.G., Suttie, J.W., Ganther, H.E. & Mertz, W. Baltimore: University Park Press.

TILLMAN, A.D., 1966. Recent developments in beef cattle feeding. Proc. Pfizer's 14th Anim. Res. Conf. pg. 7. Chicago, Illinois.

TODD, J.R., 1969. Chronic copper toxicity of ruminants. Proc. Nutr. Soc. 28, 189.

TODD, J.R., 1972. Copper, molybdenum and sulphur contents of oats and barley in relation to chronic copper poisoning in housed sheep. J. agric. Sci., Camb. 79, 191.

TODD, J.R. & GRACEY, J.F., 1959. Chronic copper poisoning in a young heifer. Vet. Rec. 71, 145.

TODD, J.R., GRACEY, J.F. & THOMPSON, R.H., 1962. Studies on chronic copper poisoning : I. Toxicity of copper sulphate and copper acetate in sheep. Br. vet. J. 118, 482.

TODD, J.R. & THOMPSON, R.H., 1963. Studies on chronic copper poisoning : II. Biochemical studies on the blood of sheep during the haemolytic crisis. Br. vet. J. 119, 161.

UNDERWOOD, E.J., 1976. Molybdenum in animal nutrition. In : Molybdenum in the Environment. Vol. 1. pg. 9. Ed. Chappell, W.R. & Petersen, K.K. New York : Marcel Dekker, INC.

UNDERWOOD, E.J., 1977. Trace Elements in Human and Animal Nutrition. 4th ed. New York : Academic Press.

VAN ADRICHEM, P.W.M., 1965. Wijzigingen in de activiteit van serumenzymen en in het LDH iso-enzympatroon bij chronische koperintoxicatie van schapen. Tijdschr. Diergeneesk. 90, 1371.

VAN DER BERG, R. & VAN DER SCHEE, W., 1973. Moglijkheden ter preventie van chronische kopervergiftiging bij schapen : toevoeging van molybdeen en sulfaat aan het krachtvoeder. Tijdschr. Diergeneesk. 90, 1371.

VANDERVEEN, J.E. & KEENER, H.A., 1964. Effects of molybdenum and sulfate sulfur on metabolism of copper in dairy cattle. J. Dairy Sci. 47, 1224.

VAN DER WESTHUIZEN, A.A. & HUGO, J.M., 1972. Die benutting van batteryhoendermis in oorwinteringsrantsoene vir jong vleisbeeste. S. Afr. Tydskr. Veek. 2, 13.

VAN ULSEN, F.W., 1972. Schapen, varken en koper. Tijdschr. Diergeneesk. 97, 735.

WARD, G.M., 1978. Molybdenum toxicity and hypocuprosis in ruminants : A review. J. Anim. Sci. 46, 1078.

WEBB, K.E., PHILLIPS, W.A., LIBKE, K.G., HARMON, B.W. & FONTENOT, J.P., 1973. Different levels of broiler litter in ewe rations. J. Anim. Sci. 36, 218, (Abstr.).

WENSVOORT, P. & HOSKAM, E.G., 1962. Kopervergiftiging bij schapen na het langdurig verstrekken van krachtvoer. Tijdschr. Diergeneesk. 87, 687.

WIENER, G. & FIELD, A.C., 1969. Copper concentrations in the liver and blood of sheep of different breeds in relation to swayback history. J. comp. Path. 79, 7.

WYNNE, K.N. & McCLYMONT, G.L., 1956. Copper - molybdenum - sulphate interaction in induction of ovine hypocupraemia and hypocuprosis. Aust. J. agric. Res. 7, 45.

APPENDIX

Appendix Table 1 Variation in liver Cu concentrations of sheep fed different quantities of dietary Cu during Trial 2.

Treatments	Liver Cu concentration (mg/kg DM)					
	1	2	3	4	5	6
	895	765	695	1457	1087	521
	1556	529	736	2246	1396	702
	1211	739	578	1994	938	1144
	1015	828	382	1542	1143	948
	1019	887	787	2079	655	856
	706	774	164	630	1268	775
	901	488	823	1751	812	1239
	804	707	572	1537	882	919
Average	1013	715	592	1730	1023	888
SE of mean	94	49	73	215	87	82

Appendix Table 2 The influence of level of dietary Mo on the GOT levels in serum of sheep on high dietary Cu and S intakes

Date of collection	15/8	21/9	27/9	9/10	1/11	28/11	6/12	13/12	8/1	24/1	19/2	14/3	2/4
						SERUM GOT (IU/dm^3)							
						No Molybdenum							
Sheep													
219	69	62	63	56	53	44	47	56	58	64	80	87	72
220	50	66	72	68	49	96	90	87	78	98	79	100	94
221	67	72	-	74	56	77	87	93	88	106	149	115	137
222	58	68	58	69	53	53	58	100	59	61	75	76	78
223	66	188	195	151	184	263	194	319	352	550	605	473	484
224	53	99	113	76	109	166	193	136	211	204	302	189	236
225	39	98	124	103	148	197	153	177	141	139	94	102	156
226	52	76	86	118	97	116	160	106	122	118	150	115	142
						Low Molybdenum							
211	52	63	59	58	45	51	55	58	52	57	64	63	67
212	97	76	77	69	58	57	58	54	53	52	69	83	79
213	53	76	71	82	62	67	71	66	76	79	107	153	145
214	130	217	216	236	163	60	114	124	263	301	151	146	115
215	71	78	76	63	62	65	64	66	78	92	115	128	87
216	79	241	185	61	149	244	203	163	159	122	93	65	127
217	54	57	56	58	56	45	45	52	44	53	65	-	-
218	68	81	74	88	65	77	58	76	93	105	109	115	117
						Medium Molybdenum							
235	76	100	97	87	76	69	82	75	69	77	91	125	107
236	58	82	89	79	68	68	61	65	77	73	76	105	78
237	71	76	83	79	91	79	90	88	65	76	93	98	120
238	50	76	79	73	58	57	71	63	48	63	78	75	75
239	65	97	102	82	80	77	77	78	73	68	83	114	91
240	55	61	62	59	56	54	60	74	64	66	69	72	64
241	60	58	61	53	55	52	52	55	44	46	60	63	59
242	65	65	64	60	-	56	58	58	81	64	65	73	57
						High Molybdenum							
227	53	73	70	60	58	54	58	58	58	67	73	69	83
228	69	76	83	82	83	70	76	61	69	77	98	91	90
229	59	58	77	72	63	60	56	47	58	88	74	75	75
230	82	80	85	70	79	73	64	53	73	76	62	91	64
231	60	164	209	182	203	82	114	87	83	141	212	363	108
232	62	80	77	70	67	65	69	57	53	59	137	93	71
233	57	64	65	56	74	64	59	57	62	52	72	92	75
234	67	80	91	-	76	48	70	66	99	98	114	140	90

Appendix Table 3 The influence of level of dietary Mo on the LDH level in serum of sheep on high dietary Cu and S intakes.

LDH (IU/dm^3)

Date of collection	27/9	9/10	1/11	28/11	6/12	13/12	8/1	24/1	19/2	14/3	2/4
No Molybdenum											
Sheep											
219	712	706	642	572	617	689	707	757	827	989	763
220	742	529	516	684	794	764	737	788	473	600	612
221	-	756	824	871	993	978	940	1019	1119	997	1086
222	858	772	693	772	790	780	868	809	862	945	771
223	1417	1085	1221	1353	1383	1714	1802	2937	2662	1924	920
224	1200	671	1058	1279	1369	1272	1524	1596	1160	1269	1500
225	1082	810	954	1137	1112	1158	1142	1112	751	867	891
226	1118	1190	1231	1361	1344	1368	1424	1439	1404	1227	1501
Low Molybdenum											
211	584	492	486	560	623	619	519	551	662	816	576
212	917	724	938	603	539	621	643	838	931	777	1425
213	888	835	735	755	744	654	742	810	862	879	1257
214	1254	1051	969	674	813	825	1164	1283	917	904	818
215	882	555	694	722	815	948	777	839	872	943	846
216	1763	883	1298	1486	1495	1400	1397	1301	872	1027	859
217	905	875	1310	735	743	766	709	776	821	-	-
218	939	900	891	961	814	942	1031	1175	1123	1235	1241
Medium Molybdenum											
235	1078	877	792	787	842	846	713	783	813	1077	821
236	809	616	593	632	587	612	629	655	636	577	618
237	901	724	792	771	834	820	745	736	806	770	753
238	1059	898	841	907	988	901	746	980	1038	1050	351
239	1066	837	832	888	943	951	883	833	907	1056	847
240	837	685	731	692	749	721	700	708	785	730	750
241	815	666	651	715	686	715	633	660	783	871	638
242	786	618	898	697	552	648	709	836	756	791	678
High Molybdenum											
227	942	645	840	719	790	835	739	841	813	713	749
228	931	703	714	732	745	746	752	844	830	717	783
229	892	693	727	664	864	717	790	1494	1029	990	760
230	1148	798	938	947	793	648	833	916	844	965	801
231	1178	892	976	693	691	784	1481	1076	1083	1385	1106
232	907	724	729	757	796	748	689	746	863	794	750
233	1005	775	887	743	801	808	1044	845	889	977	720
234	958	-	787	537	670	664	1083	984	893	791	803

Appendix Table 4(a) Liver pathology of individual sheep receiving high levels of Cu and S and different levels of Mo in their diets*

Treatment	No Molybdenum								Low Molybdenum							
Sheep number	219	220	221	222	223	224	225	226	211	212	213	214	215	216	217	218
Hepatocyte necrosis	1	2	2	1	3	3	2	3	1	2	3	2	1	1	1	2
Enlarged nuclei	1	1	1	1	2	2	2	1	1	2	1	1	1	1	1	1
Hypertrophy of Kupffer cells	1	1	2	2	2	2	2	1	1	2	2	1	1	1	1	1
Hydropic degeneration	2	2	1	1	1	0	0	2	2	3	1	1	1	1	1	1
Fatty changes	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0
Portal cell infiltration	1	1	1	1	2	1	1	1	1	2	0	1	2	2	3	1

* 0 = none, 1 = minimal, 2 = moderate, 3 = pronounced

Appendix Table 4 (b) Liver pathology of individual sheep receiving high levels of Cu and S and different levels of Mo in their diets*

	Medium Molybdenum								High Molybdenum							
Sheep number	235	236	237	238	239	240	241	242	227	228	229	230	231	232	233	234
Hepatocyte necrosis	1	1	1	2	1	1	1	1	1	1	2	1	3	1	1	2
Enlarged nuclei	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1
Hypertrophy of Kupffer cells	1	1	1	1	1	1	1	1	1	0	1	1	2	1	0	2
Hydropic degeneration	1	1	2	2	0	1	0	1	1	1	1	1	1	2	1	1
Fatty changes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Portal cell infiltration	1	1	1	1	0	1	0	0	2	2	1	2	1	1	1	1

* 0 = none, 1 = minimal, 2 = moderate, 3 = pronounced

Appendix Table 5 Kidney clearances of inulin, creatinine and urea during Trial 4.

Treatment	No Molybdenum (cm^3/min)				
Ewe number	223(a)	223(b)	224	225	226
Inulin	58,4	100,0	49,0	46,5	47,2
Creatinine	46,0	1091	22,5	298	34,4
Urea	23,1	59,3	20,8	20,9	23,7

Treatment	Low Molybdenum (cm^3/min)		
Ewe number	215	216	218
Inulin	37,7	43,3	27,8
Creatinine	72,3	44,7	24,4
Urea	44,0	30,8	12,6

Treatment	Medium Molybdenum (cm^3/min)		
Ewe number	239	241	242
Inulin	44,9	42,9	36,5
Creatinine	60,9	47,2	43,1
Urea	30,0	23,5	28,8

Treatment	High Molybdenum (cm^3/min)			
Ewe number	231	232	233	234
Inulin	33,1	51,3	62,4	52,3
Creatinine	125,4	45,5	66,3	43,0
Urea	80,0	26,4	41,8	28,2