INVESTIGATIONS INTO THE COPPER STATUS OF SHEEP GRAZING AT DIFFERENT STOCKING LEVELS

A thesis presented in partial fulfilment
of the requirements for the degree
of Master of Agricultural
Science in Animal
Science at
Massey University

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1974

ABSTRACT

A series of experiments were conducted to investigate the possible role of copper in limiting animal production at high stocking rates.

Copper and selenium were administered to 494 four-tooth Romney ewes grazing at two different stocking rates. A significant ($p < 0.01$) increase of 0.74 kg in lamb weaning weight was attributable to the supplementation of both elements. A depression in clean fleece yield ($P < 0.05$) and a small improvement in fleece crimp clarity ($P < 0.05$) were associated with copper and selenium supplementation respectively. Although both these effects reach significance at the P 0.05 level, they are considered to be due to chance.

A winter fall ($P < 0.01$) in mean plasma copper level was recorded in the high-stocked ewes.

Further investigations were undertaken in another flock of 550 Romney ewes. Factors influencing plasma, liver and wool copper were determined and relationships between plasma copper level and various fleece and body variables assessed.

Period and stocking rate were both shown to significantly ($P < 0.05$) affect mean plasma copper. No effect of lambing rank or age of ewe could be shown. A small stocking rate by age interaction was recorded ($P < 0.05$ but this is considered to have arisen by chance.

Of eighty one correlation coefficients determined between plasma copper level and various fleece and body variables, only six were statistically significant ($P < 0.05$). No biological basis could be found to account for those shown to be significant. They are considered to have arisen by chance.

No effect of stocking rate on either ewe or 'dead' lamb liver copper level could be established.

Monthly wool copper determinations indicated that the mid-winter, pre-lambing sampling was significantly depressed. No effect of stocking rate, age of ewe, or breeding rank could be established. Significant ($P < 0.05$) between-sheep differences were apparent.

Concurrent determinations of wool zinc indicated a marked depression due to both an increased stocking rate, and the onset of winter and/or pregnancy ($P < 0.01$). Older ewes had higher mean wool zinc values ($P < 0.05$).

Additional observations on the plasma samples collected in previous experiments were undertaken. Mean plasma zinc levels were found to be significantly ($P < 0.01$) depressed by both a higher stocking rate and the onset of winter and/or pregnancy.

ACKNOWLEDGEMENTS

The author wishes to thank Dr G.A. Wickham for his supervision and encouragement throughout the course of this study. My thanks are also extended to Miss L.E. Thomsen and Miss C.A. Addis for the analysis of the wool samples, as well as to the other members of the Massey University Sheep Husbandry Department's technical and farm staff who were involved in the collection of blood samples and management of the experimental flocks.

Special thanks are also extended to

Dr N.D. Grace, for the collection of the liver biopsy samples and for many helpful discussions.

Mr R.D. Anderson, who made available his computer programme for variance component estimations.

Professor R.E. Munford, for assistance with statistical analyses.

Dr R.R. Brooks, for permission to use the Atomic Absorption Spectrophotometer.

My appreciation is also extended to Mrs A.F. Barton who has accepted the task of typing this manuscript.

The work was carried out while the author received financial assistance from the Corrigil Trust.

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CHAPTER 1

INTRODUCTION

Evidence from the Massey 'CPT' flock has indicated that under high levels of stocking intensity, clean fleece weight and fleece 'quality' are depressed (Sumner, 1969; Sumner and Wickham, 1969). While fibre diameter measurement have indicated that significantly finer wool is produced under high stocking rates, visual assessments of quality number (count) would indicate coarser fleeces. It has been suggested that an apparent higher lustre may account for this depression in quality number assessment. A general reduction in wool soundness with an accompanying increase in the incidence of cotting was also recorded by these workers in the high stocked ewes.

Continued observation within this flock over recent years has confirmed the early observations (Wickham *pers comm.*).

Merino and British strong-woolled sheep grazing copper-deficient areas of Australia demonstrate reduced fleece weights, loss of crimp clarity and increased staple lustre (Bennett and Beck, 1942; Marston, 1946; Marston and Lee, 1956). Reduced tensile strength in the wool from copper-deficient sheep has also been reported by Palmer (1947) and Burley (1960).

With forage intake being appreciably reduced under the intensive stocking rate, it became apparent that the reduction in fleece weight and 'quality' could perhaps be attributable to a marginal or sub-acute deficiency of copper.

A significant selenium-copper interaction has been reported where supplementation of both these elements increased both live-weight and fleece weight in hoggets and two-tooth Romney sheep grazing marginally deficient country in Canterbury (Hill, Walker and Taylor, 1968). Responses to copper alone were also reported in hogget wool grade and two-tooth fleece weight.

The Massey University CPT flock was unavailable for supplementation studies as long-term genetical observations are being undertaken with it. In 1971, the Massey University Stress flock was founded as a duplicate of the CPT management system. This flock was made available for supplementation studies while the CPT flock was used to assess the relationship between ewe copper status and productive characters as well to assess the influence of a number of factors on ewe blood copper concentrations.

During the course of these experiments published evidence indicated that some sheep in the Manawatu area could be marginally deficient in zinc (Grace, 1972). Blood samples of ewes grazing these soils were shown to exhibit lower than average plasma zinc levels for New Zealand. Blood samples collected throughout the course of this study were also analysed, where possible, for zinc concentration.

Wool collected from monthly mid-side patch sampling of the CPT flock throughout 1968 were used to assess the importance of this pathway in copper and zinc excretion as well as assess a number of factors that may affect the level of their excretion through the fleece.

CHAPTER 2

REVIEW OF LITERATURE

COPPER IN SHEEP NUTRITION

2.1 Distribution of copper in body tissues and fluids

Copper is distributed throughout the mammalian body (Fox and Ramage, 1931). Within any particular species certain organs have been found to have consistently higher copper concentrations than others (Cunningham, 1931; Beck, 1956). The liver, brain, heart and kidneys, in decreasing order, contain the highest concentrations of copper. Intermediate copper concentrations are found in lung, intestine and spleen, and endocrine glands, muscle and bone have the lowest concentrations.

The universal occurrence of copper in animal tissues indicates that it probably takes part in a number of body functions. The presence of copper in the respiratory enzyme cytochrome oxidase being now firmly established (Waino, Wende and Shrimp, 1958).

2.1.1 Liver

Of all the body organs the adult liver contains the highest proportion and absolute amount of copper (Cunningham, 1931). Beck (1956) has determined the liver copper concentrations for a wide range of animal species. Normal adult levels differ between species although the majority fall in the range 10–50 parts per million (ppm) on a dry matter basis (DM). A few species have liver values outside this range, sheep and cattle having normal values that are much higher.

Values for "normal" mature sheep in New Zealand have been given as 171 to 1374 ppm (DM) with a mean of about 500 for two-tooth wethers and 600 for mixed aged ewes (Cunningham, 1946a; 1946b).

2.1.1(a) Influence of diet

Diet is considered the main within species determinant of adult liver copper (Underwood, 1971). Liver copper concentrations of sheep and cattle grazing copper deficient pasture have long been appreciated as reflecting reduced copper intakes (Cunningham, 1946; Bennett and Beck, 1942; McNaught, 1948). Dick (1954) fed graded increments of copper from 3.6 to 33.6 mg per day to Merino wethers. Their subsequent liver copper concentrations were found to reflect a linear relationship from 562 ppm (DM) at the lowest copper level to 2340 ppm (DM) at the highest intake. Liver copper concentrations are now considered to be the most reliable guide in the diagnosis of the copper deficient state in sheep (Underwood, 1971).

In the rat no comparable effect upon liver copper storage is seen until high dietary intakes in the order of 1 mg per day are reached (Milne and Weswig, 1968). At this level, the liver copper concentration increases rapidly due to an "apparent over-loading of the excretary mechanisms". The high concentrations obtained, however, never approach the extremely high values reported in the ovine.

The concentration of copper in the liver is also influenced by several dietary constituents which apparently alter the availability of the ingested copper. Molybdenum and sulphate are now considered to interfere with the tissue availability and storage of copper, while many elements of chemically similar structure are considered to antagonise copper absorption (Dick, 1969; Underwood, 1971; Evans, 1973). This is discussed further in section 2.2.4(b)

2.1.1(b) Influence of age

In the majority of species determined, neonatal liver copper concentrations are found to be higher than the corresponding adult values. This high concentration is found to subsequently fall during the suckling period (Cunningham, 1931). Sheep and cattle were considered notable exceptions to this. Cunningham (1931, 1946a) reported that both the concentration and total liver copper contents of ovine and bovine liver increases with age. McNaught (1948) reported a drop in the mean liver copper concentrations from 288 ppm (DM) at birth to 130 ppm (DM) at 14 days of age before a gradual rise to mature levels took place. These findings conflict with more recent evidence where newborn lambs have been shown to have higher liver copper concentrations than adults, the levels then falling with advancing age (Ryley, Harvey, Watson and Levitt, 1961; Wiener and Field, 1970). The data of the latter workers is, however, atypical; copper levels were determined only on lambs dying of natural causes while in, addition, the dams received prophylactic copper during pregnancy.

Extremely high levels of liver copper have been recorded in lambs grazing pasture containing predominately Paspalum dilatatum. This indicates the paramount importance of diet in modifying liver copper levels (Ryley et al, 1961).

2.1.1(c) Influence of pregnancy

The correlation between foetal and dam liver copper concentrations in a range of abattoir stock were found to be non-significant. Significant relationships were recorded in cattle when the dam's liver copper was below 34 ppm (DM) (Pryor, 1964).

A non-linear relationship between ewe liver copper level and size of foetuses was apparent (Pryor, 1964).

McDougal (1947) reported a rapid rise in the concentration and total foetal liver copper during the terminal three weeks of gestation in the ovine. Pryor (1964) also found a reasonable relationship between the age of foetus as indicated by "crown-rump lengths" and the dam's liver copper concentration in cattle, but no relationship could be established in sheep.

2.1.1(d) Influence of breed

Concentrations of copper in liver have been found to show significant breed variation (Wiener and Field, 1969b, 1970). Positive heterosis was present between all breed crosses except those including the Scottish Blackface. The Scottish Blackface breed and its crosses always demonstrated low liver copper values.

2.1.2 Blood

2.1.2(a) Forms and distribution

The majority of whole blood copper is distributed among four major fractions. Erythrocytes account for two fractions; 60% as erythrocuprein (erythrocyte superoxide dismutase) having a molecular weight of 35,000 and containing two atoms of copper per molecule. The remainder is considered as being in "loose association with unidentified proteins" (Evans, 1973). The role of erythrocuprein has recently been identified as preventing the accumulation of the toxic superoxide radical, a known product of xanthine oxidase and inhibitor of cytochrome C (McCord and Fridovich, 1970).

In sheep, as well as rats, dogs, pigs, cattle and man, approximately 80% of the plasma copper exists as caeruloplasmin (ferroxidase) (McCosker, 1968a; Suttle and Field, 1968; Todd, 1970). The molecular weight of caeruloplasmin is approximately 160,000 and the protein is considered to contain eight copper atoms per molecule (Underwood, 1971).

Caeruloplasmin is considered a "multi-functional" protein having been observed to oxidise adrenaline, nor-adrenaline, serotonin and melatonin in normal human serum (Osaki, Johnson and Frieden, 1966). Recent work has suggested its more essential role in oxidizing the ferrous ions entering the blood stream from the intestinal tract to the ferric state required for normal haematopoiesis (Evans, 1973).

Plasma copper not represented by caeruloplasmin is considered to be almost totally accounted for in a loosely-bound serum-albumin complex (Evans, 1973). Copper in this fraction is also termed "direct reacting copper", because of its ability to combine directly with dithizone (McCosker, 1968a). Copper in this state is now believed to be the true transport form of copper (Underwood, 1971).

2.1.2(b) Normal blood copper levels

The range of whole blood copper for normal, healthy, mature humans, rats, pigs, cats, rabbits, horses, cattle and sheep, have been given as ranging from 50 to 150 µg per 100 ml with the majority of values lying between 80 and 120 µg per 100 ml (Underwood, 1971). Specific values for the ovine have been given as 100 (Beck, 1956), 91 (Cunningham, 1946) and 98 (McCosker, 1968a).

The concentration of copper in the ovine plasma is slightly higher than in the erythrocytes (McCosker, 1968a). However, as the normal adult level of whole blood copper approaches 100 µg per 100 ml whole blood copper, plasma copper and erythrocyte copper are found to be very highly correlated in sheep with normal haematocrits (McCosker, 1961, 1968a; Bosman, 1961). Levels consistently below 60 µg per 100 ml of copper in whole blood or plasma of sheep and considered indicative of copper deficiency (Bennett and Beck, 1942; Barlow, Purves, Butler and McIntyre, 1960a, 1960b).

2.1.2(c) Influence of diet

Dietary copper strongly influences the level of copper in the blood of most species when fed in toxic or deficient amounts (Underwood, 1971; McCosker, 1968b). Low blood copper levels are known to be associated with the incidence of swayback in lambs (Bennett and Beck, 1942; Barlow *et al*, 1960a, 1960b; Wiener and Field, 1969a) and high levels in chronic copper toxicosis in sheep (Sutter, Rawson, McKeown and Haskill, 1958; Todd and Thompson, 1963).

However, unlike the rat, blood copper values in sheep and cattle only reflect copper intakes at "sub-normal" levels (Barden and Robertson, 1962; MacPherson, Brown and Hemingway, 1964; Milne and Weswig, 1968). In the ovine, normal blood copper levels are seen when the liver copper concentration range from 50 to 700 ppm (DM). Below a concentration of approximately 50 ppm (DM), the concentration of blood copper appears to fall progressively from 100 to 60 µg per 100 ml.

No general reduction in blood copper is seen below a liver concentration of 25 ppm (DM) (MacPherson _et al_, 1964; Wiener and Field, 1969b). Blood copper determinations are not recommended for use in indicating varying degrees of low copper status in sheep (MacPherson _et al_, 1964).

Sheep receiving a basal concentrate diet supplemented with 10 mg per day of copper sulphate gave no indication of a rise in blood copper concentration over a five month period (MacPherson _et al_, 1964). However, the liver copper levels were seen to range up to 700 ppm (DM). When 250 mg per day were given, blood copper values were found to frequently rise above 200 µg per ml with values as high as 1000-1200 µg per 100 ml being seen prior to, and during the haemolytic crises.

Molybdenum administration to sheep on copper-supplemented diets has resulted in increased plasma copper values while erythrocyte copper remained normal. Subsequent liver biopsy analyses indicated that the effect of molybdenum is to mobilize liver copper stores (Barden and Robertson, 1962). Recent New Zealand work has suggested that the drenching of copper-supplemented ewes with selenium will also increase their whole blood copper values while enhancing the liver storage of copper (Thompson and Lawson, 1970).

2.1.2(d) Influence of pregnancy

Many early reports indicated that pregnancy and parturition had no influence on blood copper levels in sheep (Eden, 1941; Beck, 1941; McDougal, 1947). Shear, Innes and McDougal (1940), however, concluded that blood copper levels "increased with the advance of pregnancy" while Allcroft, Clegg and Uvarov (1959) recorded an initial fall before a gradual rise in the blood copper of pregnant ewes.

More recent investigations with improved analytical techniques have demonstrated consistent variations in the opposite direction in sheep grazing pasture (Barlow _et al_, 1960a, 1960b; Butler and Barlow, 1963; Howell and Edington, 1968). The fall in blood copper was enhanced when the copper intake was consistently below that normally required to maintain normal blood copper levels but still occurred when the copper was maintained above normal by regular oral dosing with copper sulphate.

Sheep kept on constant intakes were also shown to demonstrate a fall in blood and plasma copper during pregnancy while no depression was recorded in barren ewes (Barlow, 1963). This confirmed the early suggestion that pregnancy _per se_ was causing the reduction in addition to the reduced feed intake experienced by the animals over the winter period.

Howell, Edington and Ewbank (1968) noted in grazing sheep with normal blood copper values, that in contrast to Butler and Barlow (1963), the blood copper values begin to rise at parturition, peaking approximately one week later. The latter workers having previously shown a decline persisting up until one month after parturition before rising to the premating levels. Caeruloplasmin levels were reported to reflect both blood and plasma copper levels in both of these trials.

The number of lambs born has little or no effect on the blood copper levels during the lactation period (Wiener, Field and Wood, 1969; Hayter, Wiener and Field, 1973). The rate of increase in blood copper levels between different lambing classes, however, did differ significantly between the early winter and post-partum samplings. Ewes with twin lambs showing a smaller increase in copper concentration (Wiener, Field and Jolly, 1970).

The previous years lambing class has also been shown to influence the current years blood copper changes. The movement over the same mid-winter post-partum period being in an opposite direction to that noted in relation to the number of lambs born in the current year (Wiener et al, 1970). It must be appreciated, however, that a large difference attributable to a barren class could result in a statistically significant lambing class effect without there being any "real" single versus twin differences.

2.1.2(e) Influence of age

In the neonatal ovine, whole blood copper is greater than plasma copper, there is a virtual absence of caeruloplasmin, and the level of "direct-reacting" albumin is above adult levels (McCosker, 1968a). The net result is a significantly lower neonatal blood copper level over the adult state. The transition from newborn to adult levels is considered to take approximately two days whereas in most other species recorded this period is considerably longer (Eden, 1939; McDougal, 1947; McCosker, 1961, 1968a; Howell et al, 1968). Changes in the caeruloplasmin level accounts almost totally for these changes in both blood and plasma copper (Howell et al, 1968; McCosker, 1968a).

Eden (1939) found that one year old ewes tend to have distinctly higher blood copper levels than older sheep, while Wiener and Field (1966), Wiener et al (1969) and Butler and Barlow (1963) demonstrated that young ewes pregnant for the first time have lower blood copper levels than mature ewes over the winter period.

Within mature age classes blood copper levels have been reported as increasing with advancing age, the differences being, however, statistically insignificant (Wiener et al, 1969).

2.1.2(f) Influence of breed and sire

Large differences in the blood copper levels of sheep have been found to be associated with "breed of sheep" (Wiener and Field, 1966). These breed differences have been shown to persist in spite of copper injections and variations in other factors which are known to influence the blood copper values (Wiener et al, 1969; Wiener et al, 1970; Hayter et al, 1973). Progeny of crosses involving Scottish Flackface, Cheviot and Welsh Mountain breeds of sheep have demonstrated positive heterosis (Wiener et al, 1969). However, recent work involving crosses between Finnish Landrace and Merino sheep have resulted in blood copper values intermediate between the parental values (Hayter et al, 1973).

Sheep with relatively high concentrations of blood copper maintain their values in the face of seasonal changes to a far greater extent than sheep with relatively low concentrations (Wiener and Field, 1971). This is considered to indicate that the "high group" maintains a better homeostatic control over their copper status than the "low group". Groups exhibiting low blood copper values were shown to have a higher incidence of "swayback" in their progeny.

Although no significant sire-within-breed differences could be established in the preceding investigations of Wiener and co-workers, the role of genotype in confusing the interpretations of blood copper values and the diagnosis of copper deficiencies cannot be ignored.

2.1.2(g) Influence of other factors

Blood copper concentration has been reported as having a positive association with ewe liveweight within breeds, heavier ewes having higher blood copper levels than light ewes (Wiener et al, 1969). This positive relationship contrasts markedly with a large negatative one among the breed classes determined.

Blood copper values for sheep in different "disease states" has been reported by McCosker (1968a). However, Wiener et al (1969) and Wiener et al (1970) consider the differences too small in relation to other known influences of blood copper status to be significant.

2.1.3 Wool

Wool copper concentrations of a number of New Zealand Romney sheep have been reported as ranging from 22 to 81 ppm (DM) with a mean of 44 (Healy and Zeilman, 1966). The samples in this report were derived from animals grazing a wide range of differing soil types throughout New Zealand. Similar levels have been found in a range of North American fleeces (Burns, Johnson, Hamilton, McCollock, Duncan and Fisk, 1964).

Wool copper, unlike liver copper, has been reported to increase when the ewe is grazing pastures high in molybdenum and sulphate (Healy, Bate and Ludwig, 1964; Rish, 1970). On this basis these authors suggest that wool may represent a secondary excretory pathway for copper. However, Cunningham and Hogan (1958) demonstrated a reduction in hair and wool copper when increasing dietary molybdenum levels were fed, this reduction being prevented by additional dietary copper.

2.1.4 Milk

The level of copper in milk varies with the species, stage of lactation and the copper status of the diet (Underwood, 1971). Milk copper is generally lower in concentration than the corresponding blood level which would indicate that the mammary gland has apparently no mechanism for concentrating copper in the milk (Beck, 1941).

Milk copper values from "normal" Australian Merinos are found to fall progressively from 0.20-0.64 mg copper per litre in early lactation to 0.04-0.16 mg per litre several months later. These figures also cover the ranges determined in many other species (Beck, 1941). No correlation between blood and milk copper levels has been determined. Merino sheep grazing copper deficient pasture (1-3 ppm DM) have been shown to have reduced levels of copper in their milk. However, no correlation was determined between these values and the development of anaemia (Beck, 1941).

2.2 Copper metabolism

2.2.1 Copper absorption

The copper status of many mammalian species is influenced by several dietary constituents which alter the availability of ingested copper. Many of these factors operate by altering the rate of intestinal absorption. For sheep under grazing conditions, it has been estimated that only approximately 5 per cent of the total copper intake is retained (Comar, Davis and Singer, 1948; Dick, 1954).

High dietary intakes of ferrous sulphide have been shown to depress copper absorption presumably by forming the insoluble cupric sulphide within the rumen (Dick, 1954b). Sulphur is contained in feeds in various inorganic and organic compounds and it has been shown by a number of workers that high dietary levels of inorganic sulphate or sulphur containing amino acids will produce sulphide in the rumen of sheep (Lewis, 1954; Mills, 1960; Spais, Lazaridis and Agiamides, 1968; Hartmans and Bosman, 1970. Molybdenum has been shown to promote this reduction of sulphur compounds to sulphide within the rumen.

Several elements chemically similar to copper have been shown to interact with copper availability. Cadmium, mercury, silver and zinc, because of their chemical similarity, are considered to compete for metabolic binding sites especially those concerned with intestinal absorption (Van Campen, 1970; Evans, 1973).

The greater part of copper in aqueous and ethanolic extracts of herbage appears to be in the form of low-molecular weight anionic complexes ($<$1500) (Mills, 1956; Bremner, 1970a; Bremner and Hight, 1970). The behaviour of these complexes, in terms of both their size and charge is extremely pH dependent. These extracts, and their cationic free derivatives have been shown to promote a more rapid recovery from copper deficiency in the rat than an equivalent quantity of copper sulphate (Mills, 1954, 1956). In contrast, Davis, Norris and Kratzer (1962) have shown that isolated soybean protein will adversely affect the utilization of copper.

Significant variations in the forms in which copper is present in different sections of the alimentary tract of sheep have been demonstrated by Bremner (1970b). These variations were shown to be accounted for by changes in tract pH and Bremner (1970b) considers that copper complexes could become more stable at lower pH's, facilitating copper absorption in the upper reaches of the alimentary tract. Indirect evidence in support of this hypothesis is found in the work of Dick (1952). He found that the addition of calcium carbonate to the diets of sheep will depress copper utilization presumably by depressing intestinal pH.

Differences in the mineral status of pure stands of temperate grasses has been recently documented (Patil, Jones and Hughes, 1969; Patil and Jones, 1970). Dried rations prepared from pure grass stands were shown to produce symptoms of copper deficiency as shown by depigmentations of the hair around the eyes and lack of wool crimp and colour. These symptoms were subsequently alleviated by copper supplementation of the diet. Timothy (Phleum pratense) produced the most pronounced symptoms, followed by the ryegrasses (Lolium sp). Cocksfoot (Dactylis glomerata) produced no apparent deficiency. The degree of deficiency produced was shown to parallel the levels of copper within the grasses, while in other instances it did not. In this connection it is also interesting to note that clover usually contains higher concentrations of copper than grasses (Coop, Darling and Anderson, 1953; Mitchell, Reith and Johnston, 1956).

Inter-varietal differences have also become apparent (Patil and Jones, 1970). Lambs consuming equivalent dry matter intakes of Timothy S.352 and S.48 developed symptoms of copper deficiency only on the former. These results and those discussed earlier using dried herbage become more important in view of evidence which suggests that hay curing enhances copper availability (Hartmans and Bosman, 1970). This increased availability was above that which could be accounted for by the different growth stage normally present between fresh pasture and hay, level of intake or the effect of stall feeding the hay. Paspalum hay has also been reported to alleviate some of the symptoms of copper deficiency in cattle grazing copper-deficient paspalum pasture in Northern Australia (Harvey, Ryley, Beanes and O'Bryan, 1961).

2.2.2 Copper transport

Copper entering the blood plasma from the intestine is thought to become loosely bound to serum albumin, forming the small "direct-reacting" fraction of plasma copper (Bearn and Kunkel, 1954; Bush, Mahoney, Gubler, Cartwright and Wintrobe, 1956; Evans and Carnatzer, 1970). Copper in this state is considered to be the main transport form of labile copper, it having been shown to be readily, reversibly transferable into erythrocytes, liver and other body tissues (Bush *et al*, 1956). The copper in caeruloplasmin does not appear to be so readily available for exchange or transfer with other body tissues.

Ingested copper is removed rapidly from the portal plasma and concentrates in the liver. Thereafter, a secondary increase occurs in plasma copper, accompanying the discharge of caeruloplasmin from the liver (Bearn and Kunkel, 1954).

2.2.3 Hepatic copper metabolism

The liver is the principal site of the metabolic steps involved in maintaining copper homeostatis. Copper reaching the liver is incorporated into the mitochondria, microsomes, nuclei, and soluble fractions of the parenchymal cells in proportions which vary with the age, the strain, and the copper status of the animal (cited by Underwood, 1971).

The liver is also the principal, if not the only site, of caeruloplasmin synthesis (Markowitz *et al*, 1955). In addition to its enzymatic role caeruloplasmin is thought to also function in maintaining copper homeostatis especially during neonatal development (see earlier discussion). Copper injections have been found to induce *de novo* caeruloplasmin synthesis in the adult rat, resulting in an elevation in serum caeruloplasmin and a fall in hepatic cellular copper after an initial rise (Evans, Major and Cornatzer, 1970). Indirect evidence for this model was discussed earlier, where a number of workers demonstrated close relationships between changes in the caeruloplasmin and other blood copper fractions as these fluctuated under differing copper intakes.

Bile is the major pathway for copper excretion in all species. It has been estimated that of 2-5 mg of copper ingested daily by man, 32% is absorbed, 27% is excreted in the bile, 5% is excreted directly into the bowel, while 0.5% appears in the urine (Cartwright and Wintrobe, 1964). Bile copper is found in association with amino acids, peptides and high molecular weight proteins. Protein-bound biliary copper is thought to be unavailable for reabsorption and hence copper excretion could be controlled to some extent by biliary protein excretion (Evans, 1973).

2.2.4 Antagonists of copper metabolism

2.2.4(a) Cadmium, zinc and silver

Chemically similar transition elements will produce alterations in copper metabolism that results in part from competition at the site of intestinal absorption (see earlier discussion). However, recent evidence indicates that cadmium, silver and zinc will antagonize copper metabolism within the hepatic cell, and it has been suggested that the elements inhibit caeruloplasmin activity by being incorporated into caeruloplasmin in place of copper (Whanger and Weswig, 1970, 1971). Evans *et al* (1970) have shown that both cadmium and zinc will compete with copper for sulphydryl binding sites on metallothionein from bovine liver. Hence, the interaction between copper and other chemically similar elements could result in part from competition for common binding sites both at the level of absorption and during hepatic storage.

2.2.4(b) Molybdenum and sulphate

Dick and Bull (1945) verified a lot of earlier observations which had implicated molybdenum as an inhibitor of copper metabolism in sheep. It was shown conclusively by these workers that additional dietary molybdenum would limit the proportion of copper absorbed as well as its subsequent liver storage. The degree of molybdenum antagonism has also since been found to be dependent on the dietary inorganic sulphate; high levels enhancing the molybdenum-copper antagonism (Dick, 1953).

Extensive work involving the supplementation of molybdenum and sulphate to sheep widely differing in their copper status has now conclusively demonstrated that molybdenum and sulphate will either decrease or increase the copper status of a ruminant, depending on their relative intakes, to that of copper (Dick 1954a; Wynne and McClymont, 1956).

In an experiment designed to determine the site of this antagonism Marcilese, Ammerman, Valseichi, Dunavant and Davis (1969, 1970) fed supplementary molybdenum and sulphate into sheep injected with radioactive copper. Plasma collected from the molybdenum and sulphate supplemented animals when injected into sheep kept on a basal ration resulted in a slower plasma clearance of ^{64}Cu, a significant reduction in liver copper, and a lowered plasma caeruloplasmin activity, compared to sheep injected with plasma collected from sheep supplemented with only inorganic sulphate. Other work, however, has shown that high intakes of molybdenum and sulphate do not affect the rate of injected ^{64}Cu excretion in sheep (Mills, 1961). Mills (1961) suggests that the copper-molybdenum-sulphate interaction can be explained wholly by the ability of the two anions to antagonise copper metabolism by forming insoluble cupric sulphide within the digestive tract of sheep (see earlier discussion).

A copper-molybdenum complex with a molar ratio of 4:3 has recently been formed at a neutral pH (Dowdy and Matrone, 1968a). This complex has subsequently been found to be stable *in vivo* while remaining biologically unavailable. It is excreted in the same 4:3 molar ratio when injected into sheep and demonstrates a different pattern of excretion to that seen when molybdenum is administered alone (Dowdy and Matrone, 1968b). The *in vivo* production of this complex is still uncertain; the authors consider its formation in the rumen possible. However, this has been questioned, especially in view of the presence of rumen sulphide (Hartmans, 1970). The role of sulphate in this model also remains somewhat obscure.

Recently experiments conducted in an attempt to substantiate the Dowdy-Matrone model indicated that, although the ratio of copper to molybdenum in connective tissue of rat liver approximates that predicted for the complex, that remaining in the subcellular fractions did not (Mills and Mitchell, 1962).

An alternative model resulting from observations on molybdenum and sulphate excretions studies has been proposed by Dick (1969). When high dietary molybdenum levels result in the accummulation of the element within the sheep's body tissues, it was found this could be alleviated by increasing the sulphate intake. This resultant fall in tissue molybdenum was found to be accompanied by a concomitant rise in urinary molybdenum. On this evidence Dick (1969) postulates that the sulphate and molybdate ions compete for cell membrane absorption sites consequently increasing molybdenum concentrations at these barriers. This is considered to "impede or prevent" copper transport at these barriers, especially those within the liver.

2.3 Copper deficiency and function

The role of trace elements in mammalian metabolism is now firmly established. They have been found to be primarily catalyst in the enzyme systems of the cell, their roles ranging from "weak ionic-strength effects to highly-specific metaloenzyme associations" (Schütte, 1964). In these "associations" the metal is firmly attached to a protein, with there being normally a fixed number of metal atoms per molecule of protein. The removal of the ion normally involves chemical procedures and will result invariably in the loss of the enzymic activity (Schütte, 1964). The majority of copper-containing proteins have been found to be enzymes with oxidative functions. Tyrosinase, lactase, ascorbic acid oxidase, cytochrome oxidase, uricase, monoamine oxidase and dopamine-B-hydroxylase have all been identified as copper-containing enzymes (Underwood, 1971).

A wide range of metabolic disorders have been found to be associated with a deficiency or excess of dietary copper. These include anaemia, depressed growth, bone disorders, depigmentation of hair and wool, abnormal wool growth, neonatal ataxia, impaired reproductive performance, heart failure, cardiovascular defects, connective tissue lesions and gastrotintestinal disturbances (Underwood, 1971). It would seem that as the copper reserves of an animal are depleted certain copper-dependent metabolic processes fail in their competition for the inadequate copper supply. In the sheep, specific lesions of the wool are among the first metabolic steps to be affected by a copper insufficiency (Marston, 1952).

The following is a brief discussion of some of the major disorders observed in the ovine.

2.3.1 Copper and Anaemia

Anaemia has been observed as a common expression of copper deficiency in many animals, although its morphological character varies between different species (Underwood, 1971). During copper depletion, serum copper and caeruloplasmin fall rapidly followed by a general reduction in serum iron and erythrocyte copper and eventually a dramatic reduction in red cell volume and haemoglobin content (Owen and Hazelrig, 1968). Reduced haematocrits have been found in sheep and cattle when blood copper concentrations fall below 10-12 µg per 100 ml (Beck, 1941; Marston *et al*, 1948). This anaemia has been described as microcytic and hypochromic in copper-deficient sheep and cattle and is considered identical to that seen during the iron-deficient state (Gallagher, Judap and Rees, 1956).

Extensive evidence, recently reviewed, on iron and copper metabolism would indicate that caeruloplasmin is the major link between copper and iron metabolism. Abnormalities in iron transfer at the duodenal mucosa, the reticuloendothelial system, and the hepatic parenchymal cells of the copper-deficient animals have all been related to a reduction in the ferroxidase activity of caeruloplasmin (Frieden, 1970). Although Lee, Cartwright and Wintrobe (1968) eliminated the possibility of a copper-dependent step in haem biosynthesis, many workers still consider that the excessive amounts of iron found in the normoblast suggests an inhibition of haemoglobin biosynthesis *per se* (Underwood, 1971).

2.3.2 Copper and Enzootic Neonatal Ataxia

Neonatal ataxia, or swayback, in lambs has been recognized for many years in areas of Great Britain and Western Australia (Innes and Shearer, 1940; Bennett and Beck, 1942). It has since been reported in numerous other regions of the world. It is characterized by an incoordination of the hind limbs, a stiff and staggering gait with swaying of the hind quarters. Some affected lambs are completely paralyzed or ataxic at birth and soon die, while others appear normal at birth, the condition developing progressively until locomotion becomes impossible (Innes and Shearer, 1940; Bennett and Beck, 1942).

Neonatal ataxia in Western Australia has been shown to be primarily due to a deficiency in copper intake. Ewes grazing "affected" areas develop anaemia resulting from a depletion of their copper reserves. The copper content of their liver, blood, milk and the liver copper of their progeny are also below normal values. These manifestations of copper deficiency and the accompanying ataxia in lambs responds rapidly to supplementary copper (Bennett and Chapman, 1937). Pasture and soil copper in these areas is low, the pasture containing approximately 2-3 ppm (DM) of copper, a level below that required by grazing sheep (Bennett and Beck, 1942).

In many areas of England, Wales and Scotland where ataxia in lambs has been reported, pasture copper values are low but still within the non-deficient range. Ewes grazing these areas were also found to have subnormal blood and liver copper levels (Innes and Shearer, 1940; Allcroft <u>et al</u>, 1959). Although molybdenum and sulphate supplementation has been shown to induce neonatal ataxia in experimental lambs, the concentration of both these substances in the affected pasture are considered to be within the "normal" range. It is concluded that some yet "unidentified" factor may be involved (Lewis and Allcroft, 1960).

Breeds of sheep differ in their susceptibility to neonatal ataxia (Weiner and Field, 1966; Wiener and McLeod, 1970; Hayter <u>et al</u>, 1973). In a flock comprising three differing breeds and their crosses, grazing an area with a known swayback history Wiener and Field (1966) reported an incidence of 40% of ataxic lambs in one breed, while the others exhibited incidences of only 11% and 0% respectively. The crosses had incidences approximately intermediate between the parent breeds contributing to the cross. The incidences of neonatal atoxia were subsequently shown to be closely related to the mean copper concentration in the blood of the breed classes (Wiener and Field, 1966).

Much controversy has developed over the exact pathology of neonatal ataxia (Innes and Shearer, 1940; Barlow et al, 1960). Howell (1970), in a recent review, indicated major macroscopic lesions consisting of general cell necrosis with brainstem and spinal-cord fibre degeneration. These symptoms are compatible with the description of nervous tissue degeneration reported by Bennett and Beck (1942) in Australia and Cunningham (1950) in New Zealand. A deficiency of cytochrome oxidase of the motor neurones and particularly of the large neurones of the red nucleus and spinal cord, precede the clinical disease and could well be the primary lesion (Fell, Mills and Boyne, 1965; Howell, 1970).

2.3.3 Copper and Wool Keratinization

During the early studies on neonatal ataxia in Australia it became apparent that sheep grazing copper-deficient pasture exhibit reduced fleece weights and an 'abnormal' wool style (Bennett, 1932; Bennett and Chapman, 1937; Marston and Lee, 1948; Marston, Lee and McDonald, 1948a, 1948b). The abnormal wool had been termed locally as 'steely' or 'stringy' wool and is essentially characterised by a "limp, glassy and straight appearance" (Bennett and Beck, 1942).

The deficiency first manifests itself by the appearance of secondary crimp waves which become superimposed over a deteriorating crimp in the newly grown wool. As further depletion continues the character of the fleece becomes less distinct and the wool fibres begin to emerge from the follicle as straight lustrous growths entirely devoid of crimp. These abnormalities are normally accompanied by a reduction in fleece weight (Marston, 1946). Copper supplementation of affected animals was shown to immediately restore the normal crimp pattern (Bennetts and Beck, 1942; Marston, 1946).

In many areas of Australia the first signs of copper deficiency appear in the fleece (Marston, Lee and McDonald, 1948a). Sheep grazing large tracts of Queensland produce 'silky' wool, a condition considered identical to 'steely' wool of South Australia. Sheep producing these wools have accompanying low blood and liver copper levels, but they do not produce ataxic lambs nor are they considered anaemic. The condition is also alleviated by copper supplementation (Lee and Moule, 1947).

Abnormal fleeces resulting from an insufficiency of copper have been reported in fine-woolled sheep from a number of countries. However, coarse-woolled sheep grazing swayback areas of Great Britain and peat areas of New Zealand have been reported as producing no abnormality in the wool comparable to that observed in the Australian Merino (Dunlop, Innes, Shearer and Wells, 1939; Hunter, Eden and Green, 1945; Cunningham, 1949a, 1949b, 1950, 1951). It was considered for a number of years that the Merino could be more sensitive to a low copper intake, however, Lee (1956) has reported the production of straight fibres devoid of crimp in British breeds of sheep grazing copper-deficient pastures in South Australia.

The copper-deficient lesions in the wool of Merino sheep are considered to reflect a breakdown in the physiological processes within the follicle which imparts crimp, whereas the reduction in wool production by these animals is considered to be more likely the result of an impaired appetite (Marston, 1946).

Copper-deficient Merino wool demonstrates a 35-45% reduction in tensile strength, different dyeing properties and impaired elasticity when compared to normal wool. It is slightly finer in mean diameter and processes badly (Palmer, 1949; Marston, 1946). In composition, the wool has a 30% reduction in disulphide bonds, a reduction in total sulphur content and an increased proportion of sulphyhyrl groups (Palmer, 1949; Marston, 1946; Burley, 1954, 1957, 1960). It has also been shown to contain less of a small group of highly-acidic sulphur-rich proteins with an accompanying increase in the proportions of aspartic acid, phenylalanine and leucine (Burley and Hordern, 1960; Gillespie, 1964).

The final stages of keratinization in wool fibre formation involves the conversion of sulphydril groups of prekeratin to the disulphide bonds of keratin (Matoltsy, 1962). Keratinization, normally takes place in a region approximately 100-400 µ up the developing fibre from the follicle bulb, and is normally complete within 6-8 hours (Auber, 1950; Marston, 1946). Wool-fibre keratinization in copper-deficient sheep has been shown to take place over a distance up to 1000 µ from the follicle bulb and may still not be complete when the fibre leaves the follicle (Marston, 1946). On this evidence it has been proposed that in the normal follicle, as the fibre moves up to the skin surface from the follicle bulb, the fibre proteins are aligned to a "predetermined pattern" and subsequently fixed permanently by keratinization. In the copper-deficient animal, because of the abnormal keratinization the fibre becomes disorientated before keratinization is sufficiently advanced (Marston, 1946). Copper is known to catalyse the oxidation of sulphydril groups and Catallini, Dupre and Rotilio (1969) have shown in vitro that the oxidation of cysteine to cystine involved the formation of a cysteine-copper complex. There is, however, no conclusive evidence of a copper-dependent step in the biosynthesis of the wool fibre per se (Ryder and Stephenson, 1968).

Studies of the extensibility of wool fibres from normal and copper-deficient wool, led Burley (1960) to suggest that the extra sulphydril groups and the deficit in disulphide groups in 'steely' wool can largely, but not entirely, explain their different behaviour. On this basis Burley suggests that the physical and chemical abnormalities of 'steely' wool could be more readily explained on the basis of a reduction in the concentration of the high-sulphur protein fraction. This fraction is thought to act as an "intermolecular cement" between the chains, or groups of chains, in the fibre. Deficiency of high-sulphur cement could account for both the abnormalities which are dependent on the proportion of disulphide bonds, as well as those that are not (Burley and Hordern, 1957).

The reduced concentrations of the high-sulphur fraction could also account for the reduction in cystine residues. This could be by either the loss of cystine directly from the fibre, or by the loss of potential sites for disulphide-bond formation. The latter suggestion would account well for the extra sulphydril groups found in deficient wool, which could be considered sites of potential attachment of the high-sulphur proteins (Burley, 1960).

Experiments by Gillespie (1964) support the finding of Burley (1960); however, Gillespie considers that the alleviation in the high-sulphur protein fraction accounts basically for the reduced fleece weight, whereas the reduction in disulphide bonds accounts fully for the altered chemical and physical properties of the abnormal wool. In this situation the lowered high-sulphur protein fraction is considered to be the reverse of the situation where high post-ruminal sulphur-containing amino acids give greater wool growth (Gillespie, Reis and Schinckel, 1964). The role of copper in this model is not known, although Gillespie (1964) suggests it may alter the rumen flora as in cobalt insufficiency.

2.3.4 Copper and Achromatrichia

Accumulated evidence has conclusively involved copper in the process of melanin formation in many mammalian species (Marston, 1952). Lack of pigment production in the wool of black-woolled sheep occurs at copper intakes sufficient to prevent anaemia, or other observable signs of copper deficiency (Dick, 1954a). Copper exists at the active centre of diphenol oxidases, such as tyrosinase, and when the metal is removed the activity of the enzyme is destroyed (Raper, 1928). Tyrosinase is thought to be involved in the conversion of tyrosine to melanin (Ashton, 1970). Indirect evidence for the role of copper in this model, comes from the addition of molybdenum and sulphate to the diet of black-woolled sheep. The effect of molybdenum and sulphate can occur within hours of the administration, and variation in the level of administration can result in alternating dark and light bands within the fleece (Dick, 1954a).

2.3.5 Copper and Fertility

Recently in New Zealand a significant interaction between copper and selenium in fecundity of sheep has been found (Hill, Walker and Taylor, 1968). Copper improved the incidence of twinning only in selenium-treated ewes. Neither element had any effect on the incidence of barrenness. The greater incidence of twinning among the ewes treated with selenium and copper could possibly be due to an improved liveweight and hence ovulation rate, rather than being a direct effect on reproductive physiology. Copper has not previously been associated with reduced fertility or fecundity in sheep, although in areas of Great Britain, Australia and Holland copper deficiency has long been associated with infertility in cattle (Underwood, 1971).

2.3.6 Copper and Ewe Liveweight

Severely copper-deficient sheep 'lose condition and show persistent diarrhea' (Beck, 1942; Bennetts and Beck, 1942). The depression in growth rate is considered to be related to the degree of copper deficiency, and is attributed to both a "reduced appetite and an upset in intestinal function" (Marston, Lee and McDonald, 1948b; Bennetts and Beck, 1942). Significant responses in hogget liveweight gain have been obtained when copper is administered in the presence of selenium on "marginally" deficient land in New Zealand (Hill _et al_, 1969). Animals receiving both minerals being 5% heavier than those which received only selenium.

2.3.7 Copper and Other Disorders

Although spontaneous bone fractures have been associated with copper deficiency in a number of species, only a low incidence has ever been observed in sheep and cattle (Underwood, 1971). Bennetts and Beck (1942) in Australia, Cunningham (1950) in New Zealand, and Davis (1950) in the United States have all reported isolated cases. The exact role of copper in this disorder is unknown, although some evidence suggests that copper could be involved in promoting the structural integrity of bone collagen (Underwood, 1971).

Sudden cardiac failure has been reported in copper-deficient cattle of Western Australia but the disease has never been observed in sheep grazing the same areas (Bennetts and Beck, 1942).

2.4 Copper Requirements of Sheep

The minimum copper requirement of grazing sheep is difficult to determine. It would appear that as well as breed differences in susceptibility to low copper intakes, the copper content of pasture is no real indication of its ability to supply metabolic copper.

Copper-deficient areas of Western Australia are thought to be the result of a simple deficiency of pasture copper, the majority of pasture samples averaging 2-4 ppm (DM) (Underwood, 1971). Merino sheep transferred from 'normal' to these deficient areas, ingest between 2-4 mg of copper per day and become depleted in the course of 6-8 months (Bennetts and Beck, 1942). At this point, defective keratinization appears in the wool, blood copper levels gradually fall to below 10 µg per 100 ml blood, and hypochromic anaemia results. This condition progresses slowly until the concentration of iron in the liver increases upwards of 100-fold and liver copper falls below 20 ppm (DM). At this stage of depletion ataxia in lambs is common (Marston, 1950).

Dick (1954) found that 3.6 mg of copper per day is sufficient to promote liver copper storage. From a linear-regression model estimating liver copper storage associated with gradually increasing copper intakes, Dick predicted that the minimum copper requirement of sheep could well be below 3 mg per day. This use of a linear model could, however, be an oversimplication. A curvilinear estimate would tend to increase the estimated minimum requirement. When feeding a purified diet extremely low in both molybdenum and sulphate, Dowdy and Matrone (1968b) found that blood copper levels in growing lambs could not be maintained on a copper intake as low as 1 mg per day.

Marston et al (1948a, 1948b) and Marston and Lee (1948) found that sheep grazing calcareous soils of South Australia where the pasture contains approximately 3 ppm (DM) copper, rapidly develop signs of copper deficiency when supplied with supplementary cobalt. An additional supplement of 5 mg of copper per day, equivalent to a total pasture content of some 8 ppm, was still insufficient to ensure normal blood copper levels and normal wool keratinization in all animals. Under these conditions, where there is a high consumption of calcium carbonate from the environment, with only moderate intakes of molybdenum and sulphate from the herbage, the minimum requirement of sheep is close to 10 mg per day.

Molybdenum intakes as low as 0.5 mg per day can adversely affect copper retention in sheep when the sulphate intake is high (Dick, 1954b). Cunningham (1960) has shown that in the 'peat scour' areas of New Zealand the pathological effect of molybdenum on cattle and sheep is determined by the relative amounts of copper and molybdenum in the pasture. For example, in pasture containing approximately 10 ppm (DM) of copper, about 20 ppm (DM) of molybdenum must be present before toxic effects are produced. For lower levels of pasture copper the minimum harmful level of molybdenum is lower. Excess sulphate, in the presence of 'normal' levels of copper and molybdenum have also been associated with copper deficiency and ataxia in lambs in areas of Greece (Spais et al, 1968).

In Britain, the occurrence of swayback has not been related to a low copper content in the pastures. Pasture copper values ranging from 7-20 ppm (DM) or more have been found in pastures on which the swayback has occurred (Allcroft, 1952). High lead, molybdenum, sulphate and calcium have all been implicated. Although molybdenum and sulphate are both considered to be contributing factors, no significant differences in the molybdenum content of pastures on farms where swayback is not known to occur and on those where it is enzootic, could be found (Allcroft, 1963). The 'real' antagonist of copper metabolism in these areas is yet to be established.

CHAPTER 3

SUPPLEMENTAL ADMINISTRATION OF COPPER AND SELENIUM TO ROMNEY SHEEP

3.1 Introduction

This chapter describes an experiment designed to ascertain the response to prophylatic copper and selenium in Romney ewes grazing at two different stocking rates.

3.2 Materials and Methods

3.2.1 Description of the 'Stress' Flock and its Management

The Massey University Stress flock was founded in February, 1971 from 494 two-tooth ewes purchased from four different sources. The animals were randomly allotted to two areas of pasture on the Ripley Rise property at Massey University. These areas were of 11.5 and 16.5 hectares giving stocking rates of 20 and 14 ewes per hectare respectively. The groups are subsequently referred to as the high (HSR) and low (LSR) Stress sheep.

The Ripley Rise property is situated upon a soil of the Shannon silt loam series. This series is an "intermediate" type being located between two major soil groupings, the yellow-grey earths and the yellow-brown earths (Cowie, 1972). Although the soil is considered to be of only "moderate" fertility, it is not considered to be deficient in any of the minor nutrients (see appendix I).

Pasture on the two areas grazed by the Stress ewes are composed chiefly of Perennial Ryegrass (Lolium perene) and White Clover (Trifolium repens). These areas have never been seen to produce ataxia in lambs, anaemia in ewes, or show abnormal wool keratinization.

For the duration of the experimental period the ewes within each stocking rate were allotted to the following treatment groups:

1) Copper supplemented
2) Selenium supplemented
3) Copper and Selenium supplemented
4) Control

Randomisation was restricted to ensure that ewes from each original source flock were distributed evenly between treatments.

"Cujec", a proprietary injectable copper preparation (Tasman Vaccine Laboratory) containing copper as copper glycinate was employed. Two 25 mg subcutaneous injections were given, one in March and the other in July. At these times, 6 mg of selenium as sodium selenate was also dosed as an oral drench.

The grazing system was essentially one of set-stocking throughout most of the year. The unit is entirely self-supporting in respect of feed requirements and no forage was conserved for winter feeding. All management operations other than those already discussed, were carried out as close together as was practically possible (see Table 3.1).

Table 3.1: Calendar of operations - Stress Flock, 1972

Date		Operation
March	13-17	Blood sampling of all ewes.
	17	Random allotment of ewes to treatment groups. Copper injection and selenium drenches given.
		Ewes weighed.
	31	Rams with ewes.
July	18-19	Second blood sample collected. Second copper injection and selenium drench given.
	20	Ewes weighed.
August		Lambing - All lambs tagged, weighed and birth date recorded. All 'dead' lamb livers collected.
	7	Collection of pasture samples.
November	19	Shearing - mid-side samples collected.
	30	Weaning and drafting of all lambs.
		Ewes and lambs weighed.

3.2.2 Measurement of Productive Traits

3.2.2(a) Shearing

Shearing of all the experimental animals took place on 19 November. Prior to shearing, the ewes were marked on the standard mid-side position with coloured raddle. This procedure allowed identification of the mid-side area when the fleece was on the skirting table.

After shearing the fleece was weighed and the raddle-marked mid-side sample removed and stored in a plastic bag until subsequent fleece characteristic grading.

3.2.2(b) Pre-scouring Fleece Grading

The fleece mid-side samples were assessed by the technical staff of the Massey University Sheep Husbandry Department and graded for various fleece characteristics. The descriptions, where applicable, are given in Appendix II. The characteristics assessed were:

1) Crimp clarity
2) Lustre
3) Soundness
4) Crimps per centimetre
5) Quality number

A 1 (inferior) to 9 (superior) scale is used for most grades, the system being arranged so that the distribution of grades tends to follow the normal curve.

3.2.2(c) Staple Tensile Strength

Sub-samples of the mid-side sample were conditioned in a humidity room at 65% relative humidity (RH) and 20°C for at least 48 hours (hr). The maximum load required to break the staple was then determined by a modification of the technique of Ross (1960) on a Hounsfield tensometer.

3.2.2(d) Scouring

The mid-side samples were weighed greasy after being conditioned at 20°C and 65% RH for 48 hrs. The samples were then teased and scoured using a four bowl and detergent and water scouring method. After emerging from the last bath the samples were "spun-dry" before being finally dried in a blast of hot air. The samples were again allowed to condition for 48 hrs before reweighing. The yield was calculated.

3.2.2(e) Lamb Numbers and Weights

Lambing commenced in early August. The young lambs were individually identified, tagged and weighed within 24 hrs of birth. The date of birth was recorded as number of days from August 1.

3.2.2(f) Lamb Weaning Weight

During weaning and drafting all of the lambs were weighed.

3.2.3 Determination of Ewe and Lamb Copper Status

3.2.3(a) Preparation of Glassware and Equipment

All glassware and other equipment coming into contact with materials to be analysed for trace element content were subjected to a thorough decontamination procedure similar to the method of Butler and Newman (1956)

3.2.3(b) Chemicals and Instrumentation

Elemental standards were made using AR-grade chemicals, and these solutions were stored at a concentration of 1000 ppm in 2 M HCl. Standards of lower concentration were made by dilution of a 1000 ppm solution immediately prior to use.

All quantitative elemental analyses were carried out with the use of a Varian Techtron AA5 atomic absorption spectrophotometer under the conditions listed in Appendix III.

3.2.3(c) Blood Collection and Analysis

Two methods of blood collection were employed in the course of the experiment. In the first sampling period (March) approximately 40 mls of blood was let by jugular puncture. Size 14 Luer hub needles were used, the blood being collected directly into 50 ml polypropylene centrifuge tubes containing a small amount of sodium heparin. This method was found to be laborious for the number of sheep·involved, while also possibly leading to contamination from the chrome-plated brass needle hubs (Butler, 1962). The July sample was therefore collected using a 10 ml evacuated container (Becton-Dickinson 'Vacutainer').

After collection the sample was labelled with the appropriate animal number and packed in ice. With 8 hrs of collection the samples were centrifuged at 3500 rpm for 30 mins and 3 ml of plasma withdrawn to a storage bottle. This was subsequently diluted with 3 ml of copper-free distilled and deionized (copper-free) water and stored at -10°C until required for elemental determinations.

When required for analysis, the frozen samples were thawed, shaken vigorously and assayed directly by atomic absorption spectroscopy, the method being similar to that used by Owen (1971). No allowance was made for the difference in aspiration rate between samples and standards (see Appendix IV).

3.2.3(d) Lamb Liver Collection and Analysis

Samples of liver tissue from all lambs dying within 48 hr of birth were collected and stored at -10°C. The copper contents on a DM basis were then determined in duplicate after dry ashing at 480°C for 6 hr, and finally wet ashing in a mixture of nitric and percholric acid (3:1). The subsequent ash was then taken up in 5 ml of 2m HC1 and diluted for analysis. Before determination by atomic absorption spectroscopy, the samples were allowed to stand for 30 min to permit any undissolved material to settle. Only copper was determined.

3.2.4 Pasture Copper Analysis

Random samples of pasture were collected from all areas of the experimental plots in early August. The samples were collected from above the soil level with the aid of stainless steel scissors. Duplicate sub-samples were then washed three times in copper-free water to reduce any soil or dust contamination. These were then stored at -10°C until required for subsequent analysis.

For elemental analysis, duplicate samples were taken from the thawed herbage, dried to a constant DM and ashed at 480°C for 8 hr. The samples were then prepared for atomic absorption spectroscopy as outlined in section 3.2.3(d).

3.3 Experimental Design and Statistical Methods

3.3.1 Blood Copper and Productive Traits

A factorial approach to the experiment was taken on the recommendation of Hill *et al* (1968) for trace element trials. For the ease of hand computation, 28 records of ewes bearing single lambs were chosen at random to represent each treatment group in the analyses. Responses to the treatments were tested statistically by one of the following three models:

3.3.1(a) Analysis of Variance

$$y_{ijkl} = u + a_i + b_j + c_k + (ab)_{ij} + (ac)_{ik} + (bc)_{jk} + (ac)_{ik} + e_{ijkl}$$

Where:
u = the general mean.
a_i = effect of the ith copper treatment.
b_j = effect of the jth selenium treatment.
c_k = effect of the kth stocking rate.
e_{ijkl} = the residual error which is assumed to have zero mean and constant variance.

All terms in brackets refer to the higher order interaction of the a, b and c effects, e.g. $(ab)_{ij}$ = the interaction of the ith copper and jth selenium treatments.

3.3.1(b) Analysis of Covariance (one covariate)

The model was computed as for the analysis of variance but with the additional term:

$$+ B_{yx} (X_{ijkl} - \bar{x} \ldots) \ldots\ldots$$

where:

B_{yx} is the within group regression coefficient of Y on X.

3.3.1(c) Analysis of Covariance (two covariates)

This model was computed as for the analysis of variance but with the following two additional terms:

$$+ b_{yx_1} (X^1_{ijk} - \bar{x} \,..) + b_{yx_2} (X^2_{ijk} - \bar{x}^2 ..) \ldots.$$

where:

b_{yx_1} is the within group partial regression coefficient of Y on x_1

and

b_{yx_2} is the within group partial regression coefficient of Y on x_2

Covariance analyses were employed in this model for variables where pre-experimental data was available. The technique was used to increase the precision of the estimates of the differences between means by removing inherent variations that existed prior to the treatments being imposed. However, in all the variables analysed by this technique, the influence of stocking rate was confounded also within the covariate. Hence, the interpretation of this factor within these analyses is limited to its effect on the rate of change of the variable within the period analysed.

3.3.2 Pasture and 'Dead' Lamb Liver Copper

One way analysis of variance was used to determine the significance of the stock rate differences.

3.4 Results

As stocking rate is not a 'true' main-effect of this experiment, but was included to assess the importance of any stocking rate by treatment interactions, all results are presented as sub-class means.

3.4.1 Ewe parameters

Sub-class means for both plasma copper concentration and ewe liveweight are given in Table 3.3. Included also are the means after adjustment, by covariance analysis, for the pre-supplementation values. Mean squares derived from analyses of variance and covariance are given in Table 3.2.

The initial stocking rates resulted in there being a 14% higher mean body weight in the low-stocked LSR ewes at the commencement of the trial. This difference was reduced to 3% by July, but again increased to 15% by November. The July weighing is, however, biassed by an unintentional overnight 'fast' in the LSR ewes, giving an apparent reduction in the liveweight difference expected at this time. All of these effects of stocking rate on ewe liveweight are highly significant. If the initial stocking rate effect on ewe liveweight is removed by covariance analysis, the persistent highly-significant effect of stocking rate indicates a large influence on the seasonal body weight changes. There was no liveweight response to either copper or selenium supplementation in the ewes.

The initial and winter blood copper levels of the LSR ewes were not significantly different. The small difference between stocking rates in initial plasma copper values just fails to meet the $P < 0.05$ criteria.

HSR ewes had 20% lower plasma copper values than their LSR counterparts at the winter sampling. The fall resulted in a highly significant period by stocking rate interaction. Supplementation of either or both copper and selenium in March failed to counteract the winter depression in the HSR group.

Source of variation	DF	Plasma copper March	Plasma copper July	Adjusted plasma copper July	Ewe Liveweight March	Ewe Liveweight July	Adjusted Ewe Liveweight July	Ewe Liveweight November	Adjusted Ewe Liveweight November
Stocking rate	1	578.57	10,587.50***	9,005.93***	3,003.25***	10,587.50***	745.06***	3,305.63***	235.24***
Copper	1	480.29	80.16	270.38	3.86	80.16	3.12	15.81	6.74
Selenium	1	120.07	265.79	155.71	1.97	265.79	0.31	10.50	5.09
Stocking rate x copper	1	0.07	0.64	0.50	0.38	0.64	12.39	55.50	49.26
Stocking rate x selenium	1	164.57	42.88	120.41	33.31	42.88	0.77	52.56	9.48
Copper x selenium	1	28.57	157.79	207.65	16.07	157.79	16.87	10.94	0.25
Stocking rate x copper x selenium	1	41.49	3.02	9.91	103.41	3.02	6.76	31.13	2.36
Due to regression	1	-	-	4,119.04	-	-	3,972.57	-	2,710.69
Error	215 216	158.36	149.39	130.92	255.52	149.39	5.78	29.88	17.41

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

Table 3.2: Ewe parameters - Mean squares from analyses of variance and covariance.

		Plasma Cu March (mg/100ml)	Plasma Cu July (mg/100ml)	Plasma Cu July 1/	Ewe Liveweight March (kg)	Ewe Liveweight July (kg)	Ewe Liveweight July 2/	Ewe Liveweight November (kg)	Ewe Liveweight November 3/
Low stocked	Control	89.9	86.7	86.6	50.79	47.0	46.86	51.52	48.24
	Copper	86.3	85.9	87.0	49.24	45.82	46.69	51.86	48.36
	Selenium	89.0	87.9	88.1	49.87	45.05	46.57	51.73	48.33
	Copper-Selenium	86.6	90.0	91.9	52.11	48.89	47.15	54.45	49.12
High stocked	Control	90.0	73.5	73.3	43.98	45.07	46.58	44.05	46.30
	Copper	89.1	73.4	73.5	43.98	45.82	46.69	44.89	46.26
	Selenium	95.2	73.4	71.4	43.20	45.18	46.59	44.82	46.23
	Copper-Selenium	90.3	76.1	75.00	42.55	44.93	46.55	44.05	46.00
S.E. of the means		± 2.39	± 2.31	± 2.16	± 3.02	± 2.31	± 0.45	± 1.03	± 0.79

1/ Adjusted by covariance analysis for March blood copper value ($b_{yx} = 0.35$)
2/ Adjusted by covariance analysis for March liveweight ($b_{yx} = 0.85$)
3/ Adjusted by covariance analysis for November liveweight ($b_{yx} = 0.70$)

Table 3.3: Ewe parameters - Group means, adjusted group means and standard errors (S.E.).

3.4.2 Wool parameters

Sub-class means of fleece weight, yield, tensile strength, soundness, quality number, crimp per centimetre and crimp clarity are given in Table 3.4. Mean squares derived from an analysis of variance are given in Table 3.5.

HSR ewes clipped 10% less greasy wool than the LSR ewes. The wool was significantly greater in tensile strength, had more crimps per centimetre, greater crimp clarity and reduced lustre. Selenium supplementation was associated with a significant improvement in crimp clarity.

A significant stocking rate effect on clean-scoured yield is apparent. The LSR ewes with no copper supplementation had a 4% depression in yield. There was no such difference between stocking rates in the groups given copper supplement alone and this is reflected in the stocking rate x copper interaction ($P < 0.05$). Selenium supplementation increased staple strength and soundness in the HSR ewes,however this interaction failed to reach significance at the $P < 0.05$ level.

3.4.3 Lamb parameters

Sub-class means of lamb birth weight, birth date and weaning weight are presented in Table 3.6, with the weaning weights adjusted by covariance analysis for birth date and/or birth weight. Mean squares derived from analyses of variance and covariance are presented in Table 3.7.

Stocking-rate differences were significant for lamb birth weight ($P < 0.001$), weaning weight ($P < 0.001$) and lamb birth date ($P < 0.05$). Progeny of the LSR ewes were on average 10% heavier at both birth and weaning while being born on average 4 days later than their HSR counterparts.

Source of variation	DF	Fleece weight	Quality number	Yield	Tensile strength	Soundness	Lustre	Crimps per cm	Crimp clarity
Stocking rate	1	7.57^{***}	1.58	101.29^{*}	669.27^{**}	0.76	1.29^{*}	0.300^{**}	3.76^{**}
Copper	1	0.21	0.039	42.81	42.55	0.54	0.36	0.004	0.11
Selenium	1	0.63	0.741	13.65	17.59	0.76	0.36	0.001	1.00^{*}
Stocking rate x copper	1	0.94	1.60	106.47^{*}	0.70	0.54	0.05	0.028	0.04
Stocking rate x selenium	1	0.08	0.78	0.21	120.67	3.01	0.76	0.009	0.11
Copper x selenium	1	0.19	0.93	34.28	12.29	0.36	0.11	0.001	0.54
Stocking rate x copper x selenium	1	0.00	1.20	58.62	1.45	0.11	0.22	0.005	0.54
Error	216	0.26	1.331	17.44	31.81	0.88	0.24	0.043	0.23

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

Table 3.4: Wool parameters - Mean squares.

		Fleece Weight (kg)	Quality Number	Yield (%)	Tensile Strength	Soundness	Lustre	Crimps per cm	Clarity
Low stocked	Control	3.72	46	75.55	10.7	5.21	4.89	0.90	4.61
	Copper	3.86	46	78.26	9.6	5.29	5.00	0.90	4.68
	Selenium	3.74	44/46-46	76.57	8.3	4.75	5.11	0.90	4.79
	Copper-selenium	3.98	44/46-46	78.58	7.9	5.07	5.18	0.873	4.86
High stocked	Control	3.45	44/46-46	79.58	12.9	4.93	4.93	0.95	5.04
	Copper	3.32	46	77.27	11.3	4.89	4.89	0.98	4.86
	Selenium	3.53	46	78.20	13.2	5.00	4.79	0.95	4.93
	Copper-selenium	3.53	44/46-46	79.51	12.8	5.04	4.96	0.99	5.14
S.E. of the means		± 0.96	± 0.2 of a grade	± 0.79	± 1.07	± 1.77	± 0.93	± 0.02	± 0.91

Table 3.5: Wool parameters - Means and standard errors.

Source of variation	DF	Lamb birth weight	Lamb birth date	Weaning weight	Weaning weight adjusted for birth weight	Weaning weight adjusted for birth date	Weaning weight adjusted for birth weight and date
Stocking rate	1	13.37***	1,028.57*	433.18***	105.67*	772.00***	387.79***
Copper	1	0.94	17.16	3.58	20.34	8.12	18.49
Selenium	1	0.21	0.29	2.30	7.67	2.03	5.32
Stocking rate x copper	1	1.53	297.16	33.71	5.92	3.47	0.89
Stocking rate x selenium	1	0.93	20.64	42.44	14.98	29.99	16.43
Copper x selenium	1	0.16	0.02	51.59	68.31*	52.04*	61.99**
Stocking rate x copper x selenium	1	1.56	522.16	86.13*	34.16	16.31	6.99
Due to regression	1	-	-	-	481.72	1,860.62	2,342.3471
Error	214 215 216	0.52	184.75	20.63	17.40	11.00	9.08

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

Table 3.6: Lamb data - Mean squares from analyses of variance and covariance.

		Lamb birth weight (kg)	Lamb birth date (days)	Weaning weight (kg)	Weaning weight 1/	Weaning weight 2/	Weaning weight 3/	'Dead' lamb liver copper ppm (DM)
Low stocked	Control	2.14	38.32	12.45	12.24	12.58	12.37	- (0)
	Copper	2.11	36.61	11.92	11.83	12.24	12.16	107.00 (5)
	Selenium	2.18	40.82	11.75	11.42	12.27	11.84	278.50 (2)
	Copper-selenium	2.18	36.04	13.22	12.76	13.16	12.71	139.00 (1)
High stocked	Control	1.98	34.18	11.37	11.53	11.12	11.28	139.75 (4)
	Copper	1.94	33.96	11.26	11.54	11.01	11.30	183.17 (6)
	Selenium	2.05	31.79	11.01	11.38	10.57	10.61	161.33 (3)
	Copper-selenium	1.81	37.71	10.65	11.30	10.78	11.96	144.25 (4)
S.E. of the means		± 0.45	± 2.57	± 0.39	± 0.36	± 0.29	± 0.27	

1/ Adjusted by covariance analysis for lamb birth date ($b_{yx} = -0.21$).
2/ Adjusted by covariance analysis for lamb birth weight ($b_{yx} = 2.72$).
3/ Adjusted by covariance analysis for both lamb birth weight and lamb birth date.

Table 3.7: Lamb data - Means, adjusted means and standard errors.

A highly significant copper by selenium interaction in lamb weaning weight is apparent when the weaning weights are corrected for both birth date and birth weight. Although both covariates could be influenced by the copper and selenium treatments, the data suggests that these effects, if present, are minimal and do not unduly bias the result. The adjusted means show a 6% advantage in weaning weight to the copper and selenium supplemented lambs.

Due to both a later mean birth date and lighter mean birth weight, the uncorrected weaning weights of HSR ewes show a barely significant stocking rate by copper by selenium interaction. This is considered to have no biological meaning.

'Dead' lamb liver copper levels were found to average 158 ppm (DM) and no significant treatment effects could be shown, although insufficient numbers were available for a full factorial analysis.

3.4.4 Pasture copper levels

Pasture copper values on a paddock basis are presented in Table 3.8. No paddock or group of paddocks could be shown to depart significantly from the overall mean.

Table 3.8: Pasture copper (ppm)

		S.E. of the means
General mean	13.13	± 0.76
Low stress	12.82	± 1.07
High stress	13.43	± 1.07

3.5 Discussion

The discussion of the stocking rate effect *per se* on the productive traits assessed in this experiment is limited to a comparison of the results with those of the CPT flock in recent years.

3.5.1 Stocking rate

If allowance is made for the unintentional bias in the July liveweights of the LSR ewes, the liveweight changes in the stress flock would follow an identical pattern to those reported in the CPT flock by Sumner (1969) and Sumner and Wickham (1969). This pattern is composed principally of a gradual increase in liveweight from January through until May, where the liveweight difference between stocking rates is reduced by an increased liveweight gain in the high-stocked sheep. After lambing, the high-stocked ewes lose body weight which generally remains depressed until the following autumn. However, the pre-lambing liveweight difference, and subsequent fall to weaning in the Stress flock is only approximately 13% of the corresponding values for the 1972 CPT 3 year-old ewes.

Greasy fleece weights are also clearly affected by the stocking rates imposed. The depression is, however, only 50% of the depression recorded in the CPT four-tooth ewes for 1972.

Although the fleeces obtained from the HSR ewes had a greater number of crimps per centimetre and enhanced crimp clarity, in accord with results obtained from the CPT flock, the significantly greater tensile strength and reduced lustre are not (Wickham, *pers. comm.*).

The enforced fast during the July sampling of the LSR ewes could account for their lowered staple strength. Severe nutritional stress over relatively short periods can result in marked wool break (Wickham, *pers. comm.*). Reduced lustre in the HSR is not so readily accounted for. Technical bias due to lack of objective techniques in evaluating wool characteristics can result in small differences being statistically, but not biologically, significant. No significant effect of stocking rate on wool lustre was recorded in the 1972 CPT flock.

3.5.2 Copper and selenium supplementation

Mean plasma copper concentrations at no time fell outside the accepted normal range in either sub-flock (section 2.1.2(b)). The winter depression in the HSR ewes, however, resulted in some individual sheep falling below these 'normal limits'. The failure of either copper and/or selenium supplementation to hold this depression could be due to either:

1. the level of copper and selenium supplementation was insufficient to maintain the plasma copper lever; or

2. the reduction in plasma copper concentration was not due to a deficiency of copper and selenium *per se*.

Wiener *et al*. (1969) achieved a marked increase in blood copper concentration up to four months after an injection of 50 mg of copper to ewes with blood copper levels below the normal range. Winter values in the HSR ewes were markedly higher than those reported by Wiener *et al*. (1969). It is therefore considered that 24 mg of copper should have been sufficient to maintain plasma copper if the intake of copper was depressed.

The average 'dead' lamb liver copper level of 158 ppm (DM) was just below the average of 168 ppm (DM) reported by Cunningham (1946a; 1946b) for New Zealand Romney lambs, but well below the figure of 288 ppm (DM) reported by McNaught (1948).

These results would suggest that there was no deficiency of copper in the ewes. 'Dead' lamb liver copper levels of 8 ppm (DM) are seen in copper deficient areas of Western Australia (Bennett and Beck, 1942). Pasture copper levels also indicate that unless factors antagonistic towards copper absorption and metabolism were important, there would be a sufficient copper intake to maintain normal copper homeostasis.

Reduced forage intake over the winter months resulted in the loss of body weight especially with HSR ewes (Table 3.3). A general reduction in protein biosynthesis due to both a reduced nitrogen and energy intake, as well as competition by the developing foetus, could be expected to limit the production of some body proteins.

It is suggested that a fall in caeruloplasmin synthesis is the primary cause of the reduced plasma copper level during the winter months in the HSR ewes. Caeruloplasmin accounts for up to 80% of ovine plasma copper (McCosker 1968a) and hence any variations in its levels would directly alter the plasma copper concentrations. However, the effects of nutritional stress on caeruloplasmin levels would need investigation before more definite conclusions could be drawn.

The small improvement in crimp clarity associated with selenium supplementation is probably a chance effect and should be considered in light of the discussion on technical bias when subjective appraisal is used in the evaluation of wool. A significant response in mean wool grade, however, has been reported by Hill _et_ _al_. (1969). Crimp definition and regularity were both important components in their assessment of mean wool grade.

The depression of yield at the low stocking rate is in line with the results of Sumner (1969) which show a tendency (just outside the $P < 0.05$ criteria of significance) for yield of ewe fleeces to be lower at low stocking rates. The magnitude of the difference between the non-supplemented groups and the reversal of this effect in the groups given copper alone (with the low-stocked, copper-injected group having higher yields) leads to the stocking rate x copper interaction just attaining the $P < 0.05$ level. No previous information is available to suggest that this result has any biological significance.

Copper plus selenium supplementation to the ewes resulted in a highly significant increase in the weaning weight of their lambs. Highly significant increases in growth rate of six week-old lambs receiving both copper and selenium has also been reported (Hill *et al*. 1969).

The interpretation of the interaction is complicated by the various metabolic processes which could be involved. In the present study neither mineral induced a response when given alone, indicating no 'primary' deficiency of either mineral. This is in contrast to the results of Hill *et al*. (1969) where the significant interaction was due to a secondary response to copper in lambs responding to selenium alone.

Administration of selenium to ewes will raise blood and liver copper levels of their lambs whether additional copper is given or not (Thomson and Lawson, 1970). Evidence presented by Thomson and Lawson also suggested that the supplementation of ewes with both copper and selenium will improve liveweight gains in lambs over those given selenium alone. However, the interaction was statistically non-significant. It was suggested by these workers that the increased copper status (and hence growth rate) induced by selenium was less marked at very low copper intakes.

Results of the present trial suggest that both copper and selenium are limiting growth responses to each other when given singly. This conclusion is at variance with the hypothesis extended earlier where no deficiency of metabolic copper was considered to occur. The interpretation of the copper-selenium interaction in lamb growth rate in terms of deficiencies of either elements remains obscure. If this result is not due to chance it suggests a metabolic interaction between the two elements at a higher level than has previously been suggested.

CHAPTER 4

OBSERVATIONS ON BLOOD AND LIVER COPPER IN ROMNEY SHEEP

4.1 Introduction

As the CPT flock was subject to a higher nutritional stress than that imposed on the Stress flock, but was unavailable for supplementation work, it was considered that it could be utilized in the determination of factors involved in the precipitation of any copper insufficiency. At the same time any relationship between the plasma copper levels and various production variables could be assessed.

Breed differences in tissue copper concentrations have been established in recent years (Wiener and Field, 1971) and within-breed heritabilities of doubtful accuracy have been determined (Wiener and Field, 1971b). As the sires of the experimental ewes were known, an estimate of the heritability of plasma copper concentration could be calculated.

4.2 Materials and methods

4.2.1 Description of the 'CPT' flock and its management

The Massey University CPT flock used in this experiment was grazed on two adjacent blocks of 17 and 10 hectares. At the time the observations were made these were carrying 340 and 291 ewes and hoggets respectively, giving stocking rates of 15.29 and 26.6 ewe equivalents (EE) per hectare (1 hogget = 0.6 EE). These flocks are subsequently referred to as the control and intensive CPT ewes.

All stock, except for rams, are born and bred on the unit. Castrate male lambs are sold at weaning or soon after, while all the females are reared; those not retained for breeding being discarded at random at 18 months of age. The sheep are setstocked for most of the year and the units are self-supporting in respect of feed requirements. Hay is conserved during the summer months for winter feeding.

The grazing areas are situated on a Tokomaru silt loam soil of the yellow-grey earth series (Cowie, 1972). This soil is similar to the Shannon silt loam (see section 3.2.1) with no known deficiency in any of the minor elements.

Pastures on both areas are chiefly Perennial Ryegrass (Lolium perene) and White Clover (Trifolium repens). The units have been top-dressed with 502 kg of 30% potassic superphosphate per hectare on the intensively stocked block, and 126 kg of 30% potassic superphosphate per hectare on the control block. No lime has been applied within 5 years. All fertilizer is applied in late March.

All operations on the two units were carried out as close together as was practically possible.

4.2.2 Measurement of productive traits

The measurement and recording of productive data were essentially carried out in a manner similar to that undertaken for the stress flock (section 3.2.2). However, the following minor variations were made to the fleece-grading system:

1. Mean fibre diameters were recorded.
2. Staple 'character' was substituted for crimp clarity.
3. Staple lengths were recorded.

The slight difference in the assessment of staple character and crimp clarity is outlined in appendix II. The mean fibre diameter estimations were undertaken by the airflow technique of Anderson (1954).

4.2.3 Determination of ewe and lamb copper status

Plasma isolation, sample storage, lamb liver collection and preparation, as well as the preparation of glassware, equipment and chemicals with the subsequent elemental determination, were undertaken as outlined in section 3.2.3. Blood samples were collected in both July and November in a manner similar to that outlined for the July stress flock sampling. In addition, 80 five year-old ewes were sampled with the use of 50 ml polypropylene centrifuge tubes.

Thirty of the 5 year-old ewes were also used for the estimation of ewe liver copper status. The liver specimens being collected by aspiration liver biopsy similar to the method of Dick (1952).

4.2.4 Pasture copper analysis

These were undertaken as outlined in section 3.2.4.

4.3 Statistical analysis

Only ewes with full experimental records were analysed.

4.3.1 Blood copper

A series of statistical programmes on the Massey University IBM 1620 computer were used in the analyses. These programmes could only successfully analyse two-way factorial designs. The method used is that of least-squares similar to that outlined by Harvey (1960), the model being:

$$Y_{ijk} = u + a_i + b_j + (ab)_{ij} + e_{ijk}$$

Where: u = a general mean
a_i = effect of the ith a treatment
b_j = effect of the jth b treatment
$(ab)_{ij}$ = effect of the interaction of the ith a and jth b treatments

Where: a and b refer to the main effect treatments in the following two-way analyses:

Plate 4.1: Liver aspiration biopsy

1. Age and stocking rate.
2. Age and lambing rank.
3. Age and period.
4. Stocking rate and lambing rank.
5. Stocking rate and period.
6. Stocking rate and age - lambing rank.

e_{ijk} = the residual error which is assumed to have zero mean and constant variance. In these analyses, however, it has been inflated by the absorption of the remaining fixed effects and their interaction.

4.3.2 Blood copper and productive trait associations

Product-moment correlation coefficients were calculated to determine the degree of association between productive and fleece traits of the ewes and their winter blood copper levels.

4.3.3 Lamb and ewe liver copper status

Differences between stocking rates were tested by a single classification analysis of variance. Correlation analysis was used to test for any relationship between the blood and liver copper concentrations.

4.3.4 Estimation of the heritabilities of blood copper

The paternal half sib correlation was determined from the sire and error variance components in the following model:

$$Y_{ijkl} = u + a_i + b_j + S_{ik} + (bS)_{ijk} + e_{ijkl}$$

Where: Y_{ijkl} = the plasma copper concentration of the lth ewe sired by the kth ram in the ith age group, grazing at the kth stocking rate.

u = a general mean.

a_i = effect of the ith age.

b_j = effect of the jth stocking rate.

S_{ik} = effect of the kth sire in the ith age group.

bS_{ijk} = effect of the interaction of the jth stocking rate with the kth sire within the ith age group.

e_{ijkl} = the residual error.

The coefficients used in calculating the variance components were obtained using method I of Henderson (1953). Since this method applies strictly to a random model and since age and, probably, stocking rate are best regarded as fixed effects, the variance components and the heritability will include bias. However, the bias is not considered to be serious.

4.4 Results

4.4.1 Plasma copper

Estimated main effect means and sub-class means for stocking rate and age are given in Table 4.8. Corrected mean squares derived from two-way least-squares analyses are given in Tables 4.1 to 4.6.

Mean winter plasma copper levels were 22% lower than those recorded at the spring sampling. This effect of period is highly significant.

Two way analyses of variance involving the effects of stocking rate, age and lambing class on the winter plasma copper levels gave inconsistent treatment mean squares (Tables 4.1 - 4.6). It was therefore considered that an analysis where the stocking rate and age sub-class totals were analysed as a single main effect with lambing class would give the most accurate corrections obtainable with the existing computer programmes.

The stocking rate-age effect is highly significant (Table 4.7). Multiple mean comparisons by Student-Newman-Keuls procedure (Sokal and Rohlf, 1969) indicates that the 5 year-old control ewes had significantly higher mean plasma copper levels than their 3, 4 and 5 year-old intensive counterparts (Table 4.9).

Stocking rate _per se_ resulted in the intensively grazed ewes having 7% lower mean plasma copper levels than the control ewes. Neither pregnancy, nor number of lambs born, significantly effected the mean plasma copper level at either sampling.

Table 4.1: Mean squares from least squares analysis of stocking rate and period effects on plasma copper concentration

Source of variation	DF	Mean square
Stocking rate	1	6,752.5***
Period	1	80,020.90***
Stocking rate x period	1	474.10
Residual	786	189.57

Table 4.2: Mean squares from least squares analysis of lambing and period effects on plasma copper concentration

Source of variation	DF	Mean square
Lambing rank	2	451.78
Period	1	1,050.26***
Lambing rank x period	2	336.56
Residual	786	190.36

Table 4.3: Mean squares from least squares analysis of age and period effects on plasma copper concentration

Source of variation	DF	Mean square
Age	3	590.23*
Period	1	798.60***
Age x period	3	277.63
Residual	782	196.45

Table 4.4: Mean squares from least squares analysis of stocking rate and age effects on plasma copper concentration

Source of variation	DF	Mean square
Age	3	118.07
Stocking rate	1	1,843.50***
Age x stocking rate	3	278.27*
Residual	387	106.04

Table 4.5: Mean squares from least squares analysis of age and lambing rank effects on plasma copper concentration

Source of variation	DF	Mean square
Age	3	316.10*
Lambing rank	2	446.85*
Lambing rank x age	6	145.00
Residual	383	110.01

Table 4.6: Mean squares from least squares analysis of stocking rate and lambing rank effects on plasma copper concentration

Source of variation	DF	Mean square
Stocking rate	1	178.90
Lambing rank	2	88.20
Stocking rate x lambing rank	2	101.15
Residual	389	107.60

Table 4.7: Mean squares from least squares analysis of stocking rate and age effects on plasma copper concentration

Source of variation	DF	Mean square
Stocking rate-age	7	362.43***
Lambing rank	2	170.50
Stocking rate-age x lambing rank	14	148.70
Residual	371	104.28

Table 4.8: Main effect estimated means and standard errors

	Blood copper concentration μg/100 ml			S.E.
Stocking rate	Control	84.88	(386)	± 0.70
	Intensive	79.04	(404)	± 0.69
Period	Winter	71.89	(395)	± 0.69
	Spring	92.03	(295)	± 0.69
Lambing rank	Barren	71.09	(56)	± 1.37
	Single	71.66	(296)	± 0.59
	Twin	74.24	(43)	± 1.56
Age	2 Year	81.35	(196)	± 1.00
	3 Year	82.29	(192)	± 1.01
	4 Year	84.08	(194)	± 1.01

( ) No. per subclass

Table 4.9: Age-stocking rate subclass estimates means and standard errors

Age \ Stocking Rate	Blood copper concentration mg/100 ml Control	 Intensive
2 Year	73.9 ± 1.5 ab* (48)	72.6 ± 1.3 ab (50)
3 Year	74.0 ± 1.6 ab (42)	71.4 ± 1.1 b (54)
4 Year	72.1 ± 1.4 ab (52)	69.1 ± 1.4 b (52)
5 Year	77.3 ± 1.4 a (51)	68.4 ± 1.5 b (46)

( ) No. per subclass

Note* If any two means have a letter in common they are not significantly different ($P < 0.05$).

4.4.2 Liver copper levels

Mean ewe liver copper levels were 479 ± 8 and 459 ± 7 ppm (DM) and 'dead' lamb liver copper levels were 168 ± 19 and 169 ± 10 ppm (DM) in the control and intensive flocks respectively. No significant effect of stocking rate could be shown on either ewe or 'dead' lamb liver copper levels.

4.4.3 Interrelationships between plasma copper level and prelambing liveweight

A significant within sub-group correlation is apparent between copper and ewe liveweight in the 2 year-old intensively stocked ewes. (Table 4.10).

4.4.4 Interrelationships between plasma copper level and a number of physical wool attributes

The interrelationships of winter ewe plasma copper level and a number of physical wool attributes is given in Table 4.10.

A negative association (just reaching significance) is seen between wool character and the winter plasma copper level, although only the 4 year-old control and 2 year-old intensive ewes had significant within sub-group correlations. Significant within sub-group negative associations were also found between plasma copper concentration and the tensile strength and fleece weight in the 3 year-old intensive ewes.

4.4.5 Heritability of plasma copper level

Variance components from an analysis of variance assuming all effects to be random are presented in Table 4.11. Components are given as percentages of the total variance.

$$r_{(\text{sires:ages})} = \frac{\text{Sires within ages}}{\text{Sires within ages + error}}$$

$$= \frac{3.37}{3.37 + 85.99}$$

$$= 0.0377$$

heritability being four times the intra-class correlation

$$h^2 = 4\ (0.0377)$$

$$= 0.15$$

4.4.6 Pasture copper levels

Table 4.8 lists the means and standard errors between the pastures grazed by the intensive and control CPT ewes. Neither pasture differs appreciably from the general mean of 11.04 ppm (DM).

Stocking rate	Control				Intensive				
Variable / Age	1	2	3	4	1	2	3	4	Average within Subclasses
Body weight	-0.081	-0.028	-0.051	-0.010	-0.143	0.312*	0.261	-0.073	0.0216
Fleece weight	0.069	-0.15	0.066	-0.028	0.0352	0.0576	0.291*	-0.132	0.0513
Character	-0.169	-0.177	0.051	-0.276*	-0.216	-0.228*	0.146	-0.069	-0.119*
Mean diameter	0.120	-0.187	-0.015	0.063	0.037	0.057	0.217	-0.023	0.042
Tensile strength	0.037	-0.026	-0.119	-0.140	-0.904	0.063	0.369*	-0.085	-0.-21
Lustre	0.150	-0.036	0.171	-0.105	-0.201	-0.029	-0.188	-0.290	-0.064
Staple length	-0.-37	-0.247	0.035	-0.056	-0.067	-0.008	0.164	-0.133	-0.030
Crimps per centimetre	-0.202	0.150	0.070	-0.107	0.005	0.093	0.011	0.033	-0.007
Yield	-0.019	-0.008	0.037	0.066	-0.165	0.128	-0.088	0.208	0.020

* $P < 0.05$

Table 4.10: Correlation coefficients between plasma copper concentration and a number of fleece and body variables: coefficients are given within age-stocking rate classifications as well as averaged over these classifications.

Table 4.11: Variance components - ewe winter plasma copper concentration

Source of variation	DF	Variance components
Age	3	0.06
Stocking rate	1	6.90
Sires within ages	36	3.37
Sires within ages x stocking rate	36	3.75
Residual	314	85.99

Table 4.12: Pasture copper levels

		S.E. of the means
Control	10.38 (3)	± 1.43
Intensive	11.60 (4)	± 1.24
General mean	11.04 (7)	± 0.94

Table 4.13: CPT ewe liveweights (kg)

Stocking level	Control				Intensive			
Date / Age years	2	3	4	5	2	3	4	5
January 6	42.08	49.13	51.13	48.15	33.32	35.77	37.32	38.03
February 4	42.86	50.92	53.82	52.55	33.23	37.62	37.49	35.02
March 13	40.96	47.74	50.45	48.30	34.67	37.87	38.03	34.99
April 20	42.59	49.85	53.86	52.39	35.53	39.34	39.81	37.39
May 11	43.74	50.42	53.88	52.28	35.36	39.54	38.94	36.32
June 16	41.92	50.10	53.22	51.07	35.95	40.61	40.17	37.46
July 25	44.05	51.66	54.27	51.14	36.55	41.64	39.91	37.33
November 29	48.19	50.97	51.22	50.11	37.89	40.96	38.20	33.65

4.5 Discussion

4.5.1 Plasma copper levels

At neither of the two sampling times did any of the sub-groups mean plasma copper levels fall below 60 µg/100 ml, the generally accepted level, indicative of copper deficiency in sheep (section 2.3). Although the spring levels were close to the level of 91 µg/100 ml reported by Cunningham (1946) for 'normal' New Zealand sheep, the winter average of 71.89 µg/100 ml was markedly depressed.

A winter depression in ovine blood copper have also been reported by Barlow et al. (1960a, 1960b), Butler and Barlow (1963) and Howell and Edington (1968), who suggest that the depression is due principally to both a reduction in copper intake due to reduced forage availability, as well as a specific effect of pregnancy (Barlow, 1963). These reported falls were apparent even when regular oral dosing with copper sulphate was undertaken. This is in contrast to the present study, and that of other workers, where no specific effect of pregnancy or number of lambs born could be established (Beck, 1941; Eden, 1941; McDougal, 1947; Hayter et al. 1973). Different breed and nutritional planes could account for the different results, however, clarification will require additional investigations.

High plasma copper levels in the five year-old control ewes is primarily responsible for the 'apparently' significant age by stocking rate interaction. Why this class of ewe should have markedly higher than average plasma copper levels is not apparent. In the absence of supporting evidence it is considered to be due to chance. A proportion of the variation accounted for in the interaction is also due to the fall of plasma copper with age within the intensively-stocked ewes. Ewe liveweights (Table 4.13) suggest that the nutrition of the ewes falls with increasing age. However, mean age difference in plasma copper level within this class of sheep fail to reach the 5% criteria of significance. Age has previously been reported to only contribute to variations in blood copper concentration in young sheep pregnant for the first time while no significant variation is apparent in ewes 2 years-old and over (Eden, 1939; Wiener et al, 1969; Hayter et al, 1973).

The difference between stocking rates is not considered to be seriously biased by the 'apparent' significance of the age by stocking rate interaction. The high value in the 5 year-old controls has resulted in some exaggeration of the difference, but if allowance is made for this the difference and the absolute levels are markedly lower than those reported in the control ewes of the stress flock. The principal cause of the between stocking rate and flock differences appears to be differences in nutritional stress imposed, as evidenced by the ewe liveweights (Table 4.13).

4.5.2 Liver copper levels

Mean liver copper levels of 479 and 459 ppm (DM) for the control and intensively-stocked ewes respectively, are within the normal range, although below the mean of 600 ppm (DM) reported by Cunningham (1946a, 1946b) for mixed age New Zealand Romneys.

The average 'dead' lamb liver copper levels are close to the mean value reported by Cunningham (1946a, 1946b) but below the value of 288 ppm (DM) given by McNaught (1948) for New Zealand Romney lambs.

These levels would indicate that the copper intake is unlikely to be limiting copper storage within the CPT ewes. Levels of liver copper below 100 ppm (DM) are considered to result from lowered copper intakes, while levels below 15 ppm (DM) have been reported in copper deficient areas of Western Australia (Bennett and Beck, 1942). No between stocking rate differences could be established indicating that the additional nutritional stress of high stocking does not significantly limit copper storage.

Although the 'dead' lamb liver copper levels were below the mean level reported by McNaught (1948), they are not so low as to be suggestive of copper insufficiency. Levels of 8.0 ppm (DM) have been reported in deficient areas, while levels of between 120-300 ppm (DM) are seen in the livers of lambs in healthy areas of Western Australia (Bennett and Beck, 1942).

The normal liver copper levels found in the CPT flock support the hypothesis extended earlier in section 3.5.2, that is nutritional stress is restricting plasma copper levels it is independent of the copper intake.

4.5.3 Liveweight of ewe

In contrast to the results of Wiener et al. (1969) within-class correlation coefficients between blood copper and ewe liveweight were found to be statistically insignificant except for a small positive relationship in the 2 year-old intensive ewes. As this coefficient just meets the $P < 0.05$ criteria it is unlikely that it would have any biological significance.

4.5.4 Interrelationships of copper and physical wool attributes

The small negative association found between blood copper concentration and character in the 4 year-old control and 2 year-old intensive ewes is not consistent with the known role of copper in crimp formation and definition (section 2.3.3). A small negative association between tensile strength and fleece weight in the 3 year-old intensive ewes is also apparent. As all of these associations just meet the $P < 0.05$ criteria for significance, and as no biological evidence is available to suggest any antagonism between copper and these attributes, it is suggested that their statistical significance is due to chance.

The general lack of association between copper and the physical wool attributes measured would tend to indicate that plasma copper never reached a level such that keratinization was affected to any measurable extent. If metabolic or available copper is identified with the free or "unbound" plasma copper fraction, the suggested fall in caeruloplasmin could not be considered to affect wool growth and development.

4.5.5 Heritability of plasma copper

Breed differences in the concentration of copper in the blood of sheep have been reported by Wiener and Field (1966, 1970, 1971a), Wiener, Field and Wood (1969) and Hayter et al. (1973). Estimates of heritability of blood copper were estimated by Wiener and Field (1971a) by calculation of both the paternal half-sib correlation and by use of the dam-offspring regression. The former estimate was of low accurancy due to the small numbers of animals involved, while the value of 0.41 for the latter method is based on the assumption that the mineral concentration in the lamb is genetically perfectly correlated ($r_g = 1$) with the concentration for the same animal when adult. As such, it also assumes that maternal effects on the trait under calculation are small (Falconer, 1961). Although Wiener and Field (1971a) suggest that these effects are small for blood copper, the value reported casts some doubts as to the validity of these assumptions. Gross between-breed differences have been reported and evidence has been presented suggestive that different genes may be controlling plasma copper levels in different breeds (Hayter et al, 1973). Estimates of heritability within breeds may also differ.

The heritability reported in the present study suggests that very little of the variation is attributable to genotype. A reasonably large sire by stocking rate interaction is also apparent with only a small proportion of the variation in the estimates of ovine plasma copper being accounted for by the model.

4.5.6 Pasture copper

The pasture copper value of 11.04 ppm (DM) would suggest that unless high forage molybdenum, or other antagonistic factors are present, ample copper is available to supply dietary needs (section 2.4).

CHAPTER 5

THE CONCENTRATION BY MONTH OF COPPER AND ZINC IN THE WOOL FROM ROMNEY SHEEP

5.1 Introduction

Variation in the copper and zinc contents of wool have been reported for a range of soils in New Zealand and U.S.A. (Healy and Zielman, 1966; Healy *et al*, 1964; Burns *et al*, 1964). Large between locality differences were reported by all workers, these differences being principally considered to be due to variations in soil elemental concentrations.

Wool elemental levels have been suggested as a possible source of readily analysable tissue for large scale trace element surveys to determine areas of possible trace element deficiency (Healy *et al*, 1964). The investigation reported here is an attempt to determine the importance of a number of factors which could influence the level of copper and zinc in the fleece of Romney sheep.

5.2 Materials and methods

Wool samples collected during 1966-67 from the CPT flock were used in the course of this experiment. These samples consisted of eleven consecutive, monthly, mid-side samples which were collected from each sheep in the course of an experiment designed to determine the effect of increased stocking rates on wool growth.

The collection and handling of the samples, as well as a full discussion of the flocks management during this period, has been presented by Sumner (1969) and Sumner and Wickham (1969). However, the more important aspects in relation to the present experiment are given here.

5.2.1 Collection, handling and storage of samples

The management of the flock during the collection period was similar to that outlined in section 4.2.1.

Mid-side patch samples were collected from the 2 and 4 year-old ewes at 28 day intervals from December 29, 1966. No samples were taken on August 31, 1967 (sampling 9) due to lambing being in progress.

After collection the greasy wool samples were conditioned in a humidity room (20°C and 65% RH) for 48 hr. The first two samplings were scoured using the four bowl detergent and water scouring procedure, but in subsequent samplings a sequence of organic solvents (5% ether : 95% ethanol) and cold water were used. Little agitation was applied in either method. Clean fleece production, fibre diameter and fibre length estimations were made (Sumner, 1969). Subsequently the samples were stores in individual brown paper envelopes.

In the present investigation 3 samples were randomly chosen to fill the appropriate clases of Table 5.1.

Table 5.1: Ewe fleece sampling groups

		Control	Intensive
2 year-old	Single bearing	3	3
	Twin bearing	3	3
4 year-old	Single bearing	3	3
	Twin bearing	3	3

5.2.2 Preparation of wool samples for elemental determination

Samples of wool weighing 200 mg (at 20°C and 65% RH) were rinsed in copper and zinc-free water for 24 hr, after which any discernible foreign matter was removed as the samples were draining on black netting cloth. The samples were ashed at 480°C for 8 hr in a muffle furnace, the ash being then dissolved in 5 ml of 2 M HCl and diluted for subsequent atomic absorption spectroscopy. Both copper and zinc were determined, the instrument being operated under the conditions outlined in appendix III.

5.2.3 Statistical analysis

Treatment effects were tested statistically by an analysis of variance according to the following model:

$$\begin{aligned} Y_{ijklm} &= u + a_i + b_j + c_k + d_l + (ab)_{ij} + (ac)_{ik} + (ad)_{il} + (bc)_{jk} \\ &\quad + (bd)_{jl} + (cd)_{kl} + (abc)_{ijk} + (adb?_{ijl} + (bcd)_{ikl} \\ &\quad + (abcd)_{ijkl} + F_{ijkl} + (dF)_{lm} \end{aligned}$$

Where: u = the general mean
a_i = the effect of the ith stocking rate
b_j = the effect of the jth breeding rank
c_k = the effect of the kth age
d_l = the effect of the lth period
F_{ijkm} = a random effect of the mth sheep in the ith stocking rate, in the jth breeding rank, in the kth age group.

All terms in brackets refer to the higher order interactions of these main effects, e.g.

$(ab)_{ij}$ = the interaction of the ith stocking rate with the jth breeding rank

Sheep and period effects were tested against the sheep by period interaction, while all other main effects and their interactions were tested against either the sheep by period interaction or the sheep effect depending on whichever was the larger mean square.

Least-significant-difference (LSD) analysis was used to test which of the period means were significantly different from sampling one.

5.3 Results

5.3.1 Wool copper

Main treatment means are given in Table 5.3(a)(b). Individual period means averaged over breeding rank are presented in figure 5.1. Mean squares derived from an analysis of variance are given in Table 5.2.

A significant effect of period ($P < 0.01$) was found subsequently, by comparisons of the individual period means to result from sampling eight being lower than sampling one ($P < 0.05$). No other significant treatment effects could be established.

Significant between-sheep ($P < 0.001$) variation is apparent.

5.3.2 Wool zinc

Main treatment means are given in Table 5.3(a)(b). Individual period means averaged over breeding rank are presented in figure 5.2. Mean squares derived from an analysis of variance are given in Table 5.2.

Significant effects of period ($P < 0.001$), age ($P < 0.01$) and stocking rate ($P < 0.05$) are apparent. Two year-old ewes had 5% lower wool-zinc levels than their four year-old counterparts, while the intensively grazed ewes had 4% lower mean wool zinc levels than the control ewes. Between period comparisons indicated that samplings 4, 5, 6 and 8 were lower than sampling one ($P < 0.01$).

Table 5.2: Mean squares from analysis of wool copper and zinc concentration

Source of variation	DF	Mean squares for copper	Mean squares for zinc
Stocking rate (SR)	1	56.89	209.18*
Age	1	5.78	307.67**
Breeding rank (BR)	1	49.57	63.03
Period	10	178.75**	116.94***
SR x age	1	9.49	2.01
SR x BR	1	27.12	58.70
Age x BR	1	740.24	21.31
Period x age	10	74.41	57.44
Period x BR	10	74.51	23.74
Period x SR	10	22.09	29.36
SR x age x BR	1	1.80	96.21
SR x age x period	10	71.82	21.51
SR x BR x period	10	113.57	37.78
BR x age x period	10	37.66	17.70
SR x BR x age x period	10	92.99	34.98
Sheep within stocking-rate, age and breeding rank	16	609.71***	38.83
Residual	160	69.65	26.28

Table 5.3: Wool copper and zinc concentration main effect means

(a)

		Wool copper concentration ppm	Wool zinc concentration ppm
Stocking rate	Control	33.3 ± 4.5	104.5 ± 1.5
	Intensive	34.3 ± 4.5	100.0 ± 1.5
Age	2 year-old	33.5 ± 4.5	99.5 ± 1.5
	4 year-old	34.0 ± 4.5	104.8 ± 1.5
Breeding rank	Single	34.3 ± 4.5	103.5 ± 1.5
	Twin	33.3 ± 4.5	101.0 ± 1.5

(b)

Sampling period	1	2	3	4	5	6	7	8	10	11	12
Wool copper concentration ppm	36.3	32.5	37.3	35.0	31.0	33.8	33.0	25.5*	34.3	32.5	33.3
Wool zinc concentration ppm	108.8	108.0	105.8	97.3**	94.0**	93.3**	103.8	98.3**	102.5	105.0	107.0

* Significantly different from sampling 1 $P < 0.05$

** Significantly different from sampling 1 $P < 0.01$

Copper $LSD_{0.05} = 10.1$ zinc $LSD_{0.01} = 9.8$

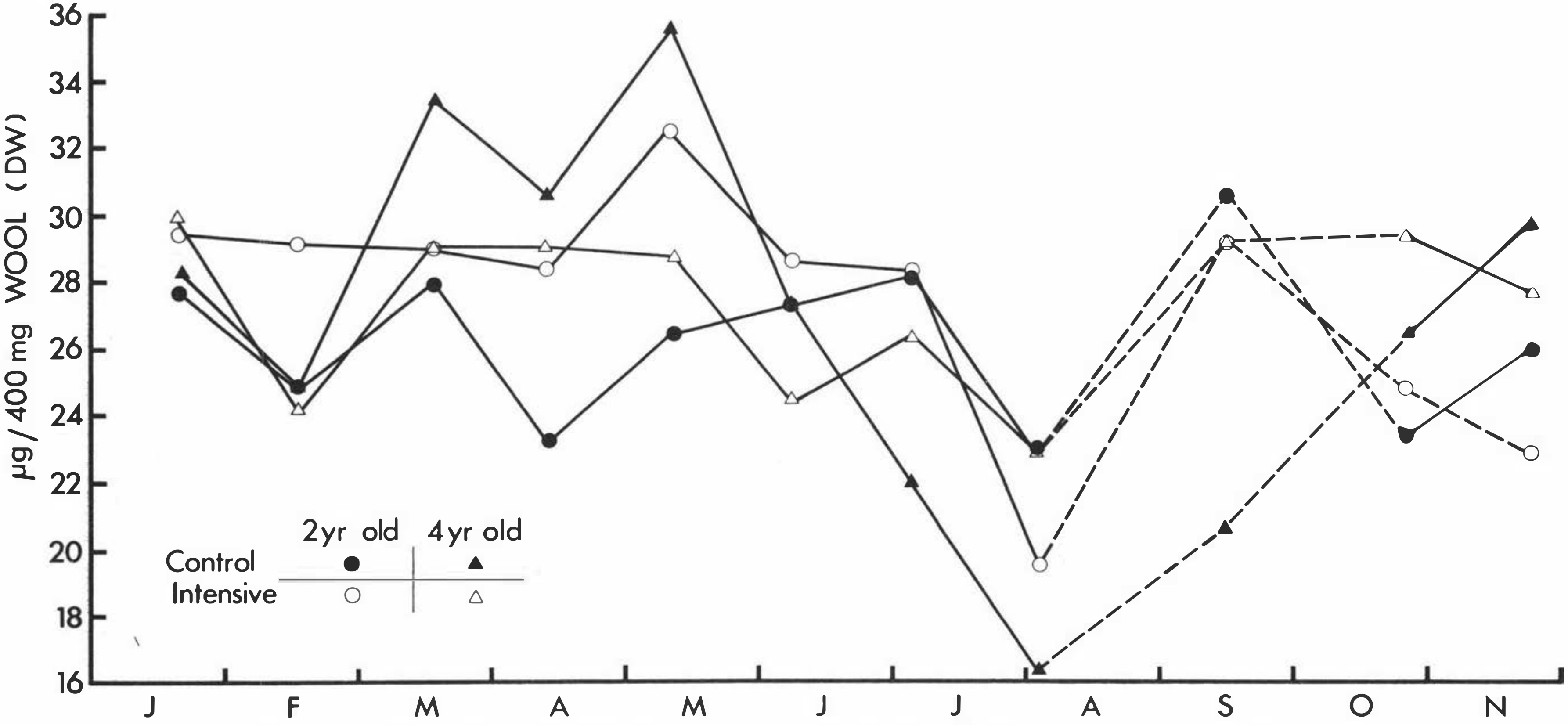

FIG. 5.1 WOOL COPPER CONCENTRATION PER 28-DAY PERIOD FROM A MID-SIDE PATCH

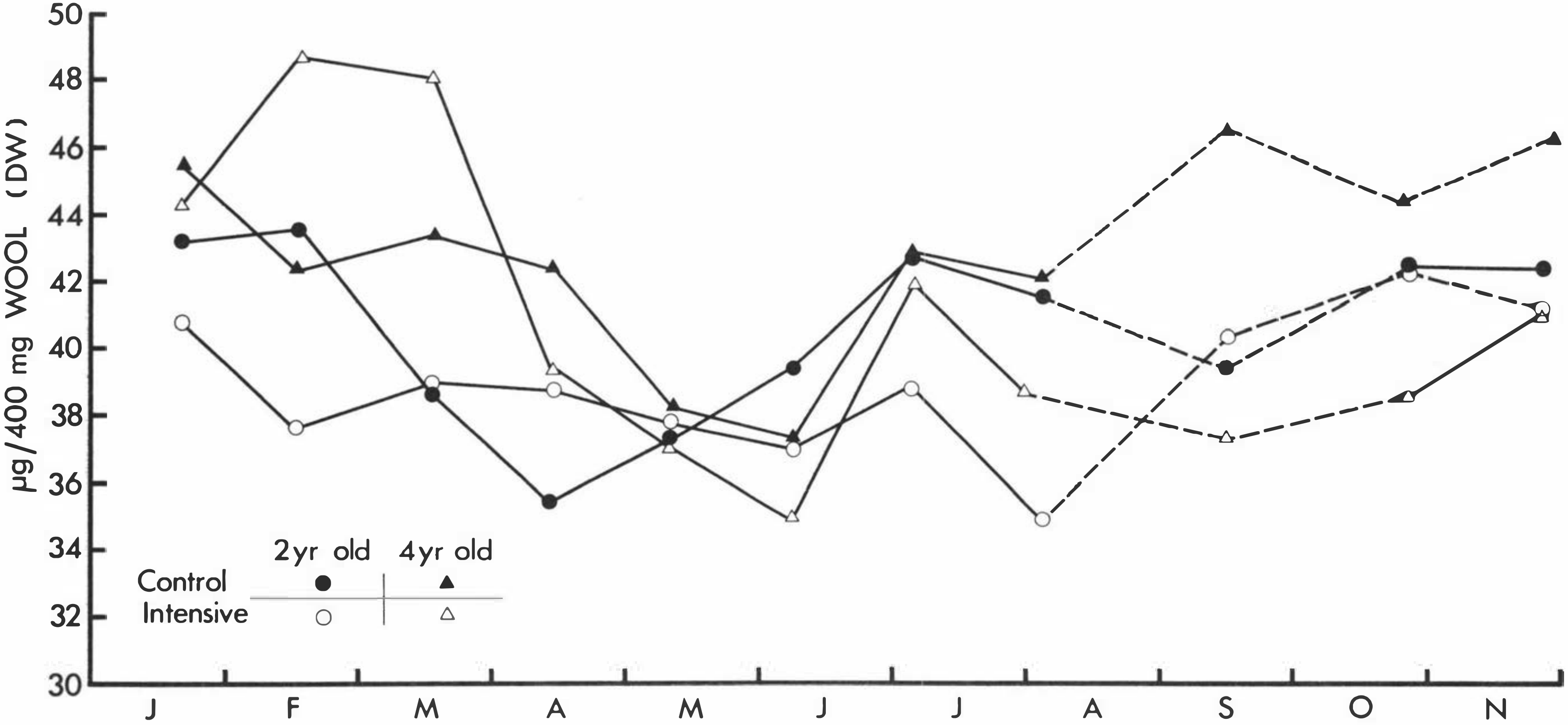

FIG. 5.2 WOOL ZINC CONCENTRATION PER 28-DAY PERIOD FROM A MID-SIDE PATCH

5.4 Discussion

5.4.1 Wool copper

The mean wool copper level of 34 ppm is within the range of 22-81 ppm reported by Healy and Zeileman (1966) for sheep grazing a range of New Zealand soils.

The significant between-sheep differences strongly suggest that genotype could be an important determinate of wool copper concentration within the Romney breed. Although only a small proportion of the variation in plasma copper could be attributable to sires in the present study (section 4.4.4), large between-breed differences in tissue and organ trace element contents have been established (Wiener and Field, 1966, 1971a, 1971b; Wiener et al, 1969, 1970; Hayter et al, 1973).

If genotype is an important factor in the determination of wool copper, some of the observed variation between sheep grazing different soil types in the U.S.A. and New Zealand could be accounted for by between-flock or locality genetic differences.

Although sampling eight is the only period significantly lower than sampling one, general tendencies are still apparent. A depression in wool copper is evidenced from June through until August corresponding to the depression in wool growth reported by Sumner (1969) and Sumner and Wickham (1969). The limitation to wool copper level was probably due to a low level of nutrition in late winter and early spring.

A number of workers have shown that the dietary copper intake will influence the level of copper in the wool of sheep and hair of cattle (Cunningham and Hogan, 1958; van Kotveld, 1954, 1958 cited by Cunningham and Hogan, 1958; O'Mary et al, 1970). Large dietary differences in either the copper or molybdenum intakes are necessary to affect wool copper. Copper intakes varying from 6.15 to 9.71 mg/day do not significantly affect mean wool copper (Kapoor et al, 1972.

No prolongation of the low levels of wool copper into September were observed in the intensive ewes as was reported for wool growth in these sheep (Sumner, 1969; Sumner and Wickham, (1969). Large between-sheep differences, coupled with the small number of samples analysed, does not necessarily indicate that they do not exist.

No significant effects of stocking rate, age or breeding rank were observed on wool copper concentrations. It can only be assumed that the extra nutritional stresses due to

1. increased stocking rate,
2. competition from an additional foetus during pregnancy, or
3. greater demands from a still maturing ewe,

do not place demands heavy enough on the metabolic copper reserves to result in reduced wool copper levels. It is difficult to reconcile that the nutritional stress of a reduced winter intake should cause a decline in wool copper, while an increased stocking rate does not. It could be accounted for by increased soil intake in high stock ewes similar to that documented by Healy (1968, 1972) for cobalt, selenium and iodine, or that the wool copper level is affected by seasonal physiological fluctuation.

5.4.2 Wool zinc

The mean wool zinc level of 102 ppm is within the range of 96-102 ppm reported by Healy and Zeileman (1966) for a range of New Zealand soils. Unlike wool copper concentration, there were no significant differences between sheep suggesting little influence of genotype on fleece zinc excretion within Romney sheep.

A highly-significant effect of period ($P < 0.001$) where wool zinc levels fall in April and remain depressed until August is seen. The initial April fall is one month earlier than the seasonal fall in wool production and three months earlier than body weight changes would suggest a marked fall in the plane of nutrition

(Sumner, 1969; Sumner and Wickham, 1969). Seasonal variations in the availability and/or supply of pasture zinc could presumably be responsible for the depression. However, the lack of any information on the forage mineral status does not allow a more conclusive interpretation.

The anomolus deviation in the July wool zinc level, although possibly the onset of a late winter-early spring rise, can only be adequately accounted for by some more drastic alteration in zinc nutrition. Without more specific information on the zinc intake it can only be suggested that it could be possibly due to an onset of winter hay feeding.

With the wool zinc levels being depressed for at least four months of the year it is suggested that the ewes were in a state of zinc insufficiency, at least during the late autumn-early winter period. If this is in fact the case, the effects of age and stocking are readily interpreted.

The lowered wool zinc level in the intensively grazed ewe would be a direct result of a lowered forage intake. Why even lower levels were not recorded in the July-August samples is even more surprising. As suggested with copper, a higher ingestion of soil under the high stocking rate could to some extent compensate for a lowered forage intake.

The lower mean wool zinc level in the 2 year-old ewes could possibly be due to a greater biological demand in this age class. Two year-old sheep having still not reached mature body size; metabolic demands could be expected to be greater.

CHAPTER 6

PLASMA ZINC CONCENTRATIONS IN ROMNEY SHEEP GRAZING AT TWO STOCKING RATES

6.1 Introduction

During the course of the investigations into blood copper (Chapters 3 and 4) it became apparent that many areas of New Zealand carried flocks with relatively low plasma zinc levels (Grace, 1972). A zinc level of 57.7 ug/100 ml of plasma was reported for ewes grazing Tokomaru silt loam. This value is below the New Zealand and North Island averages and well below the value of 100 µg/100 ml reported for optimal growth in sheep (Ott, Smith, Stob, Parker, Harrington and Beeson, 1965). However, other workers have reported a level of 45 µg/100 ml below which growth is retarded in lambs (Mills, Dalgarno, Williams and Quartermain, 1967).

6.2 Materials and methods

Blood samples collected in the course of the earlier experiments were used for the estimation of plasma zinc levels in Romney ewes from both flocks grazing under two different stocking rates. All of the samples collected in the 'Vacutainers' were heavily contaminated with zinc (see appendix V). Thus only the initial Stress flock samples and subsequent samples collected in 50 ml polypropylene centrifuge tubes were suitable for analysis in the present study.

The handling of the plasma samples and subsequent instrumentation were identical to those outlined in section 3.2.4.

Effects of both stocking rate and season were tested statistically by variance analysis using the following model:

$$Y_{ijk} = u + a_i + b_j + (ab)_{ij} + e_{ijk}$$

Where:

u = the general mean

a_i = is the effect of the ith stocking rate

b_j = is the effect of the jth period

$(ab)_{ij}$ = is the interaction of the ith stocking rate with the jth period

e_{ijk} = the residual error which is assumed to have zero mean and constant variance.

6.3 Results

6.3.1 Stress flock

Mean plasma zinc concentration for both the autumn and winter sampling periods, as well as between stocking rates, is given in Table 6.1. Mean squares derived from an analysis of variance are given in Table 6.2.

Table 6.1: Stress flock - Plasma zinc concentration mean squares

Source of variation	DF	Mean square
Stocking rate	1	1,696.91***
Period	1	3,123.82***
Stocking rate x period	1	41.02
Error	216	123.68

Table 6.2: Stress flock - Plasma zinc concentration means and standard error

Low stocked	High stocked	Autumn	Winter	S.E.
64.2	58.6	65.2	57.6	± 1.50

Both season and stocking rate had highly significant effects on mean plasma zinc concentration. A 9% reduction in plasma zinc concentration of the HSE is seen, while a depression of 12% in the mean plasma zinc level is seen at the winter sampling. Both effects are additive.

6.3.2 CPT flock

Mean plasma zinc concentrations for both the winter and spring sampling periods and for the two stocking rates is given in Table 6.3. Mean squares derived from an analysis of variance are given in Table 6.4.

An 11% reduction was recorded in the intensively stocked ewes compared to the controls, while a 17% depression in the winter sampling is apparent. Both of these effects are highly significant.

Table 6.3: CPT flock - Plasma zinc concentrations mean squares

Source of variation	DF	Mean square
Stocking rate	1	768.80 ***
Period	1	2,000.00***
Stocking rate x season	1	72.20
Within groups	76	58.25

Table 6.4: CPT flock - Plasma zinc concentration means and standard error

Control	Intensive	Winter	Spring	S.E.
58.45	52.25	50.35	60.35	± 1.71

6.4 Discussion

Zinc levels in the plasma removed from some of the Stress flock sheep at winter sampling could have been affected by the selenium and copper supplementations described in Chapter 3. However, no such tendency is obvious in the data and no reports of interactions of zinc with these elements in biological systems have been encountered.

The mean plasma zinc concentrations (Stress flock 61.4 µg/100 ml, CPT flock 55.4 µg/100 ml) are close to the value of 57.7 reported by Grace (1972) for sheep grazing pastures on Tokomaru silt loam soil.

Stocking rate and time of sampling were probably related to plasma zinc levels through the effects of lower levels of availability of all nutrients at the high stocking rate and during winter.

The 10% difference between the Stress and CPT flocks is probably also a reflection of a lower overall nutritional level in the CPT flock, but different fertilizer treatments and differences between the Shannon silt loam and Tokomaru silt loam soils, could also be implicated.

The level of 47.3 µg zinc per 100 ml in the plasma of the CPT intensive ewes at winter sampling is only slightly above the level associated with limitation of lamb growth (Mills et al, 1967).

CHAPTER 7

GENERAL CONCLUSIONS AND SUMMARY

Investigations into the possible role of copper in limiting animal production at high stocking rates were undertaken in 1972.

The conclusions were:

1. Winter plasma copper levels of 87.9 and 79.0 ug/100 ml were recorded in the high-stocked treatments of the Stress and CPT flocks respectively. These levels are not indicative of copper deficiency.

2. Pasture copper levels of approximately 100 ppm (DM) are sufficient to supply an adequate copper intake assuming no major antagonistic influences.

3. Liver copper levels of both ewes and 'dead' lambs were close to reported "normal" New Zealand values.

4. A 24 mg copper injection and/or oral dose of 6 mg of sodium selenate failed to maintain the spring plasma copper levels in the HSR ewes over the winter. This and previously stated observations suggest that a deficiency of copper *per se* is not the cause of the winter fall.

5. A small (0.74 kg) advantage in lamb weaning weight was attributable to the combined copper and selenium supplements. No other 'real' responses in ewe productivity could be established from their supplementation when given singly or together.

6. In the CPT flock mean winter plasma copper levels were 78% of their spring values, while the intensively-grazed ewes were 7% lower than their control counterparts.

7. No effect of either age or lambing rank could be established on mean plasma copper levels.

8. No 'real' relationships could be established between plasma copper concentrations and a number of production variables. This would suggest that copper levels never reached a state where ewe production was limited.

9. By use of the intra-sire correlation, the heritability of plasma copper concentration in Romney sheep was estimated as 0.15. This value is lower than other, less-accurately determined, estimates.

10. No effect of either stocking rate, age or breeding rank could be established on the mean wool copper concentration throughout a 12 month period. However, the August pre-lambing sample was significantly depressed in comparison to other monthly values. Significant between-sheep differences in wool copper concentrations were apparent.

11. Significant effects of stocking rate, age and period were found on mean wool zinc concentrations. An average depression of 13% was seen in the April, May, June and August samples. No significant between-sheep difference or effect of breeding rank could be established.

12. Both stocking rate and time of sampling were found to significantly effect the mean plasma zinc levels of the Stress and CPT ewes.

13. Intensively-stocked ewes in both flocks had mean plasma zinc levels 11% and 9% below their control stocked counterparts, while winter depressions of 17% and 12% were recorded in both flocks over their spring and autumn values respectively.

14. The mean winter plasma zinc level of 47.5 ug/100 ml recorded in the intensively stocked CPT ewes at the winter sampling is only slightly above the level of 45 ug/100 ml associated with limitations to lamb growth (Mills _et al_, 1967).

15. Evidence reported here would suggest that except for a possible slight improvement in lamb growth rate, neither copper or selenium was limiting animal production in the experimental flocks.

16. Plasma zinc levels suggest that an insufficiency of metabolic zinc could be limiting animal production at high stocking rates in both the Stress and CPT flocks. Zinc is known to be involved in wool keratinization as well as lamb growth (Mills _et al_, 1967). The sensitivity of wool zinc to various "nutritional" stresses would tend to support this hypothesis.

APPENDIX I

Physical and chemical soil parameters

	Shannon silt loam	Tokomaru silt loam
Parent material	Loess	
Internal drainage	Slow	
Nutrient status	Moderate/low:P Ca Medium:K	
Pasture response	P, K and Ca	
Description	Weakly leached moderately gleyed yellow-grey earth	Weakly leached moderately to strongly gleyed yellow grey earth
Rainfail	1020 - 1270 mm	1020 - 1270 mm

Spectrographic analysis (approx) for reference soil Marton silt loam

Macroelements %							
Al	8	11	12	13	13	10	11
Fe	3.5	4	4.2	4.1	4	4	5
Ca	1.5	1.5	1.3	1	1	1	0.9
Mg	0.7	0.9	0.9	0.8	0.65	0.65	0.65
Na	1.2	1.3	1.5	1.2	1.3	1.4	1.3
K	0.8	0.8	0.8	0.8	1	0.8	0.9
Microelements p.p.m.							
Zr	270	250	300	300	300	350	300
Cr	18	20	20	30	20	20,	20
Ni	4	6	6	6	8	6	4
Co	4	4	3	3	4	2	2
Mn	980	1200	250	150	150	150	200
Mo	2	2	3	2	3	2	3
Ga	9	10	12	12	12	12	15
V	80	100	100	100	100	100	100
Cu	15	15	15	20	20	20	20
Ba	800	900	900	800	1000	900	800
Sr	530	500	400	200	150	300	300
Ti	2500	3000	3500	3500	4000	3000	3000
Loss on ign. %	11	7	4	5	6	3	3

APPENDIX II

Description of the fleece characteristic grading system

Below are listed descriptions for the fleece characteristic grading system. Standards were kept where possible. Measurement technique is given in cases of measurable characteristics.

Quality number

Bradford quality numbers were used. For the statistical analysis the numbers were coded;

e.g. 46 = 5
46/48's = 6
44/46 = 4

These were reconverted back to quality numbers for presentation in the tables.

Staple crimp clarity

A scale from 1-9 was used.

1. crimp practically indistinguishable
9. extremely even and pronounced crimp.

Fleece character

Fleece character grades depended upon the staple size, freedom from tippiness and hairiness, as well as staple crimp evenness and clarity. Character grades ranged from 1-9 and was similar to the 1-7 grading system used by the Ministry of Agriculture and Fisheries.

Crimps per inch

The number of crimps over the whole staple were counted, divided by the staple length (cms) and converted to crimps per cm by tables. Estimated on an average staple.

Handle

Assessed without regard to quality number with the sample screened from the view of the assessor. Line first surveyed to obtain samples.

1. Extremely harsh
2. Markedly harsher than average
3. Clearly harsher than average
4. Slightly harsher than average
5. Average handle
6. Slightly softer than average
7. Clearly softer than average
8. Markedly softer than average
9. Extremely soft.

Lustre

Graded with no reference to fineness.

1. No lustre (Merino)
2. Very slight lustre
3. Low 2nd demilustre
4. 2nd demilustre
5. Low 1st demilustre
6. High 1st demilustre
7. 2nd lustre (English Leicester)
8. 1st lustre (Lincoln)
9. Like coarse Lincoln.

Soundness

Test carried out on a "standard" sized small staple. If unable to be broken staple is divided into half.

1. Much of fleece lost
2. Very weak
3. "Break" present
4. Slight pull to break
5. Good pull to break
6. Slight pull to break ($\frac{1}{2}$ staple)

7. Good pull to break ($\frac{1}{2}$ staple)
8. Good pull to break ($\frac{1}{4}$ staple)
9. Sound ($\frac{1}{4}$ staple).

Staple length

Average staple selected, measured to nearest centimetre unstretched but flattened.

Quality number

Bradford quality numbers.

APPENDIX III

Atomic absorption spectrophotometer operating conditions

	Zinc	Copper
Acetylene flow	3	$3\frac{1}{2}$
Air pressure	15 psi	15 psi
Flame	Reducing	Reducing
Lamp current	6 mA	3 mA
Slit width	300 u	50 u
Wave length	2,139 A^o	3,247 A^o

APPENDIX IV

Aspiration of ovine plasma diluted with copper-free water (1:1) and copper-free water

Plasma (1:1)	Copper-free water
5.6 ml/minute	5.1 ml/minute
5.5 " "	5.2 " "
5.6 " "	5.3 " "
5.6 " "	5.2 " "
5/6 ml/minute	5/2 ml/minute

The 0.4 ml/minute difference in aspiration rates between diluted plasma and copper-free water was not considered large enough to bias the calibration of the instrument.

APPENDIX V

Trace element contamination of Vacutainers and polypropylene centrifuge tubes

Samples of five tubes were filled with a 5% solution of copper-free hydrochloric acid and analysed for copper and zinc levels after increasing storage intervals. Tubes were collected at random throughout the blood samplings.

No significant degree of copper contamination was recorded. However, levels of between ten and twenty ppm of zinc was recorded in the vacutainers, the level increasing with storage time.

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