AN EVALUATION OF THE EFFICACY OF ORALLY ADMINISTERED COPPER GLYCINE COMPLEX, COPPER AMINO ACID CHELATE, COPPER SULPHATE, A COPPER OXIDE WIRE PARTICLE BOLUS AND A COPPER EDETATE INJECTION IN NEW ZEALAND DAIRY COWS

A thesis presented in partial fulfillment of the requirements for the degree of

Masters in

Animal Science

Massey University, Palmerston North, New Zealand.

Shaun Christopher Balemi

2008

ABSTRACT

This thesis set out to examine the difference in efficacy of the most commonly used copper supplements in New Zealand dairy herds. There is limited information on copper supplementation in New Zealand dairy cattle in the area of chelated (organic) verses sulphated (inorganic) supplements and this study was designed to provide more information to the New Zealand dairy industry.

Sixty non-pregnant mixed age Friesian dairy cows, on the basis of liver copper concentrations, were randomized into 6 groups of 10 animals so that each group had the same mean liver copper concentration. The treatments were Group 1, non-supplemented control; Group 2, 150mg copper/day as copper glycine chelate drench; Group 3, 150mg copper/day as copper amino acid chelate drench; Group 4, 150mg copper/day as copper sulphate drench; Group 5, 20g copper oxide wire particles administered as a bolus and Group 6, 100mg of copper, as calcium copper edetate, administered as a subcutaneous injection on days 1 and 58. The duration of the study was 116 days and the cows were fed baleage, with limited access to pasture. On days -5, 14, 28, 58, 86, and 116 after supplementation, liver samples were obtained by a biopsy technique and blood from the coccygeal vein for copper determinations.

The mean initial copper concentration in the liver of the cows used in this study was 827 (SE 109) μmol/kg fresh weight (FW). The mean liver copper concentration of the cows in the control group decreased significantly ($P<0.05$), from 827 (SE 109) μmol/kg FW on day 1 to 554 (SE 114) μmol/kg FW on day 116. Over days 58 to 116 the mean liver copper concentration of the copper glycine chelate, copper amino acid chelate, and copper sulphate groups where significantly ($P<0.05$) greater than the non-supplemented control group. The combined means over the 6 sampling events showed that the group supplemented with the copper glycine chelate had significantly ($P<0.05$) greater liver copper concentration than the group supplemented with copper sulphate (1064 versus 910μmol/kg FW). The mean liver copper concentrations of the group which received the copper oxide wire particle boluses were consistently greater than the control group;

however a significant difference was only achieved at the day 58 sampling. The group injected with copper edetate achieved a significant rise in liver copper concentration on day 86 after being injected on day 58. However, when the group was injected at day 1 no significant rise was achieved at day 14, 28, or 58. The copper supplements had no effect on serum copper concentrations. Despite the large variation (SE 109) in initial liver copper status between the cows, this did not influence the amount of copper stored in the liver regardless of the copper supplement used. The data was analysed in two groups, cows with lower liver copper (553 μmol/kg FW), and cows with higher liver copper (1050 μmol/kg FW), and there was no difference between the two groups in response to the copper treatments.

The initial liver copper concentration of the cows was high. A copper intake of 150mg copper/day was effective in increasing the copper concentration of the liver of dry non-pregnant New Zealand dairy cows. As an oral supplement, copper glycine chelate was more effective in increasing liver copper concentrations than copper sulphate. Overall the oral supplements (copper glycine, copper amino acid chelate, and copper sulphate) were more effective in increasing liver copper concentration than the copper oxide wire particle bolus and the twice given 2ml copper edetate injections. The copper oxide wire particle bolus maintained liver copper concentrations at 843μmol/kg FW which is an adequate liver copper concentration. Therefore in this situation where liver copper concentrations where adequate prior to supplementation the bolus did provide enough copper. This study indicated that in order to maintain liver copper concentration in dry non-pregnant New Zealand dairy cows, on a low copper diet, a 2ml injection may have to be given every 45 days.

ACKNOWLEDGEMENTS

I would like to thank Prof Dave West and Dr Neville Grace for their commitment to directing and aiding me in the completion of this thesis, without their support the writing of this thesis would not have been possible.

I would like to thank my Father and Mother for the support, both moral and financial, which they have freely given.

I would like to thank Robin Whitson, Stefan Smith, various 5th year vet students, and the rest of the LATU staff for the management of the animals used in the trial, for direction and commitment to the drenching, and for their help on the sampling days.

I would like to thank the Taupo Animal Welfare Health and Veterinary Society Inc, Agvance Marketing Ltd, and Massey University for the financial assistance they generously provided making this research project possible.

I would like to thank Nicolas Lopez-Villalobos for his direction in the statistical analysis of the data.

I would like to thank all the postgrads on the 3rd floor for their willingness to give help and answer questions.

Lastly I would like to thank the Lord God for His provision, favour, and blessing, for without Him none of this would have been possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The trace element nutrition of dairy cows in New Zealand has for many years been seen as an area which must be addressed in order to maintain production, health, and reproductive performance. Copper has been given particular attention as it was one of the first trace elements to be found lacking in New Zealand livestock. Ira Cunningham first recognised copper deficiency in livestock with copper responsive disorders such as "swayback" in lambs and peat scours in cattle in the early 1940's. Since then further investigation has shown that many of New Zealand's pastures cause copper deficiency in grazing livestock.

New Zealand livestock gain the majority of their copper from the pasture they graze. The concentration of copper in pasture ranges between 2 and 20mg/kg DM (ppm), with normal levels seen as being around 8-10mg/kg DM (Howell & Gawthorne, 1987a). However many of New Zealand's pastures contain less than 8mg Cu/kg DM and this along with a high level of antagonists present in many of New Zealand's soils cause widespread copper deficiencies in grazing livestock.

There are two types of copper deficiency which occur in livestock; deficiency related to low copper levels in the pasture, and deficiency due to antagonists in the soil and pasture which reduce the absorption of copper by livestock (Smith, 1972). The first report of copper deficiency associated with antagonists in livestock was by Ira Cunningham in 1954, where cattle dosed with a high level of molybdenum where found to be copper deficient (Cunningham, 1954).

The effects of a molybdenum induced copper deficiency in New Zealand have been reported as poor live-weight gain, bone and nervous disorders, reduced milk production, diarrhoea, post parturient haemoglobinuria, reproductive disorders, and peat scours (Cunningham, 1954; Ellison, 1992). Therefore the impact of copper status on the New Zealand agricultural industry is highly significant.

Molybdenum has a large influence on copper deficiency seen in New Zealand as many New Zealand soils and pastures contain high levels of molybdenum. Reports of copper

deficiency in New Zealand livestock caused by high iron, zinc and sulphur levels in pasture have also been made (Lee et al., 1999). Iron, zinc, and sulphur are also antagonists along with molybdenum, interfering with copper absorption in the digestive tract causing copper deficiency.

Copper deficiency in livestock has been identified through-out New Zealand (Cunningham, 1957; Dowling, 1997; Lee et al., 1999) but livestock grazing in regions such as Northland, Hawke's Bay, Waikato, and Southland have been found to suffer from widespread copper deficiency.

The impact of copper deficiency on the New Zealand agricultural industry is marked and well known. However the methods of reducing the effects of copper deficiency in New Zealand livestock through copper supplementation are not. Therefore, greater knowledge of the efficacies of the many copper supplements available on the market today is needed.

This thesis will investigate the efficacy of three oral copper supplements (copper glycine complex, copper amino acid chelate, and copper sulphate), a copper oxide needle bolus and a copper injection in New Zealand dairy cows. The differences between the supplements will be discussed and conclusions drawn on the effectiveness and practicality of each.

CHAPTER 1

Literature Review

FUNCTIONS OF COPPER

Copper plays an essential role in the health and performance of livestock through the function and concentration of essential enzymes (table 1). There is a close relationship between dietary copper concentration and enzymatic activity (Sharma et al., 2005).

The major enzymes to which copper contributes are; ceruloplasmin, monoamine oxidase, superoxide dismutase, cytochrome oxidase, lysl oxidase, peptidylglycine α, dopamine β and tyrosinase (table 1) (Sharma et al., 2005; Underwood & Suttle, 2000).

These copper dependant enzymes are linked to functions of copper in the mammalian body; these will be described in greater detail in the pages following.

Connective tissue development

Copper is essential in the development of connective tissue in young ruminants through the enzyme, lysl oxidase (Howell & Gawthorne, 1987b). This enzyme catalyses the formation of the intermolecular cross linkages in elastin and collagen giving strength to both the elastin and collagen formed during growth (Howell & Gawthorne, 1987b; Underwood & Suttle, 1999). Joint abnormalities, and cardiovascular problems have been found in animals which are deficient in copper as a result of poor connective tissue development (Howell & Gawthorne, 1987b; Underwood & Suttle, 2000).

Bone development

The role copper plays in lysl oxidase formation also influences bone development in mammals (Underwood & Suttle, 2000). Lysl oxidase is an essential enzyme in the mineralization of cartilage and formation of bone collagen; therefore it may have a direct

Table 1. The major copper dependant enzymes and there functions in the mammalian body (Underwood & Suttle, 2000).

Enzyme	Function	Significance
Ceruloplasmin	Fe^{2+} - Fe^{3+}, copper and iron Transport	Anaemia
Cytochrome *C* oxidase	Terminal electron transfer, CNS Function	Anoxia k
Lysl oxidase	Desmosine X-linkages in connective tissue	Aortic rupture, joint disorders, bone strength, mobility
Peptidylglycine α-amidating monooxygenase	Elaboration of numerous biogenic molecules	Appetite
Copper-zinc superoxide dismutase	Dismutation of O_2^- to H_2O_2	Antioxidation
Tyrosinase	Tyrosine to Melanin	Depigmentation
Dopamine *B* monoxygenase	Catecholamine metabolism	Behaviour

effect on the strength of the bone formed (Underwood & Suttle, 2000). Bone strength is dependent on the presence of "cross-links" in the collagen formed and copper deficiency can result in a reduction of these "cross-links" (Howell & Gawthorne, 1987b). Bone deformities in young livestock due to copper deficiency can be reversed if adequate copper supplementation is performed (Howell & Gawthorne, 1987b).

Central nervous system development

The condition known as "swayback" or "enzootic ataxia" is a common central nervous system disorder in lambs, and is the result of a copper deficiency in the pregnant ewe giving birth to a copper deficient lamb (Howell & Gawthorne, 1987b; Underwood & Suttle, 2000).

Copper influences the central nervous system via the enzyme cytochrome *c* oxidase which is essential in the development of the myelin sheath around the nerves thus essential in the development of the central nervous system (Howell & Gawthorne, 1987b). Cytochrome *c* activity is significantly reduced in the brain of lambs suffering from swayback and copper deficiency (Underwood & Suttle, 2000).

Appetite

The copper dependant enzyme, peptidylglycine-α-amidating monoxygenase, is important in the formation of the appetite regulating hormones gastrin and cholecytokinin (Underwood & Suttle, 1999). This enzyme is essential in maintaining the balance of appetite regulating hormones which are important for driving hunger and thus important for livestock performance and production.

Erthropoiesis

Copper is required for the distribution of iron throughout the body, iron absorption and the release of iron, through its influence over the production of the enzyme ceruloplasmin (Williams et al., 1983). It is thought that long term copper deficiency causes anemia due to reduced iron release from various sites throughout the body leading to a decrease in the iron available for erthropoiesis and various other functions (Williams et al., 1983).

Suttle & Jones (1987) showed evidence of the effect copper status has on anemia (through Heinz body count and haemoglobin concentration). In animals which were copper deficient Heinz body counts were significantly higher and haemoglobin concentrations were significantly lower than in animals which had an adequate copper status (Suttle & Jones, 1987). After copper supplementation Heinz body counts were significantly reduced and haemoglobin concentration increased in sheep (Suttle & Jones, 1987).

Immunocompetence

Copper has been found to influence the normal functioning of the immune system of livestock (Howell & Gawthorne, 1987b; Underwood & Suttle, 2000). The immune system has two approaches to the defence from pathogens; specific and non-specific immune cells (Minatel & Carfagnini, 2000; Underwood & Suttle, 2000). In copper deficient animals the concentration of specific immune cells is reduced, meaning immune response to pathogen challenge is reduced (Underwood & Suttle, 2000). Suttle *et al.*, (1989) showed that lambs which were, deficient in copper had a significantly lower immune response when challenged with various pathogens; whereas lambs with a normal copper status had a significantly greater immune response to pathogenic challenge.

The enzyme superoxide dismutase has been found to influence phagocyte (immune cell) function in ruminants (Suttle & Jones, 1989; Xin et al., 1991). A reduction in superoxide dismutase leads to an increase in oxidative damage to phagocytes (neutrophils) reducing their effectiveness in engulfing and removing pathogens entering the body (Suttle &

Jones, 1989). Xin (1991) found that copper deficiency significantly reduced superoxide activity in cattle, over an 8 month period, when compared to cattle with an adequate copper status.

The correlation between copper superoxide dismutase, neutrophil function and immune function shows strong support for copper being important in the immune function of livestock.

Pigmentation

Copper is essential in the pigmentation of hair, and skin in the mammalian body (Howell & Gawthorne, 1987b). The conversion of tyrosine to melanin by the copper containing enzyme tyrosinase is the pathway by which pigmentation occurs (Howell & Gawthorne, 1987b; Underwood & Suttle, 1999). Therefore in the instance of copper deficiency tyrosinase concentration is reduced causing depigmentation in the ruminant and is seen as an early sign of copper deficiency in ruminants (Howell & Gawthorne, 1987b).

Antioxidant function

There are several different pathways in which copper influences antioxidant function in the mammalian cell. The different antioxidant pathways influenced by copper are; superoxide dismutase, impairment of iron metabolism, ceruloplasmin and metallothioneins (Al-Gubory et al., 2004; Klotz et al., 2003; Underwood & Suttle, 1999).

The most commonly known antioxidant linked to copper is superoxide dismutase (SOD), this enzyme is the catalyst which activates the oxidation of reactive oxidative species O^- into hydrogen peroxide. Then the enzyme glutathione peroxidase (GPx) converts the hydrogen peroxide into water (Klotz et al., 2003). The reaction which occurs during this process is; $2O_2^- + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2 \xrightarrow{GPx} H_2O$ (Klotz et al., 2003). Copper is essential in the formation of superoxide dismutase, with zinc being the catalyst to its oxidizing function.

METABOLISM OF COPPER IN RUMINANTS

The metabolism of copper in ruminants involves the processes of: absorption, transport, storage, utilisation and excretion (figure 1).

From the portion of copper which is consumed in the diet a percentage is absorbed in the gut and passed into the blood, the remaining copper which was not absorbed in the gut is passed out of the animal in the faeces. The blood then transports the copper to the liver, bone, soft tissue, and various other organs in the body.

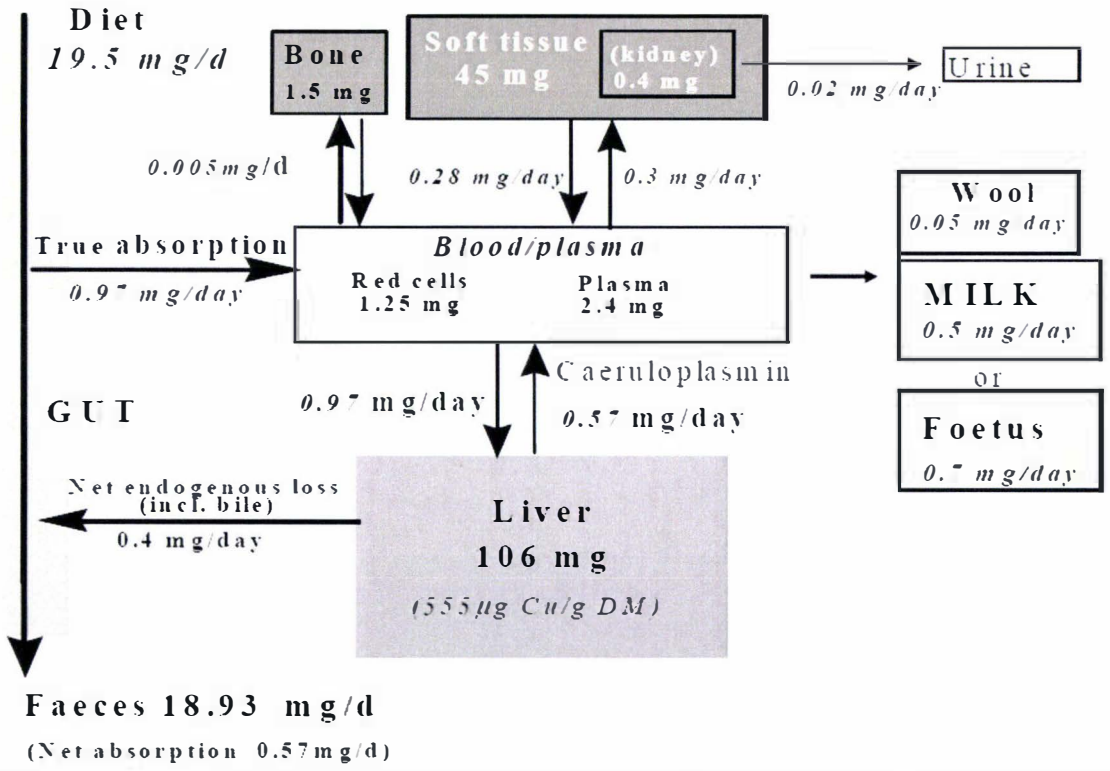


Figure 1. The processes involved in copper metabolism in a 50kg Romney ewe at maintenance, showing where copper enters the animal in the diet, a proportion of that copper is absorbed into the blood and is then circulated around the body with a proportion of that copper being used by the body, used for production, stored in the liver, and excreted via the urine (Lee & Grace, 1997).

Different portions of the copper in the blood is then stored in the liver, passed out in the urine, passed out into the lower gut in the bile, or utilised for maintenance, growth, wool, milk production, and the growth of the foetus.

Each of the major metabolic processes involved in copper metabolism will be discussed further in the following sections.

Absorption

Copper absorption in ruminants ranges from <1.0% to 10% (Spears, 2003), which is low when compared to the 30% to 40% copper absorptive range of mono-gastric animals (Wapnir, 1998). The low relative absorption of copper by ruminants is due to the interaction of copper with other factors in the rumen. The copper absorption range of a pre-ruminant calf (having a non-functioning rumen) is between 70% and 85%. However once the calf is weaned and is a fully functional ruminant its copper absorption drops to between 1.0% and 10% (Spears, 2003). The amount of copper lost from the diet of ruminants, via the faeces, ranges between 66% and 97%, which accounts for the largest proportion of un-utilised copper in the diet. Losses via the urine were found to be less than 8% (Grace, 1975).

Absorption of copper from the digestive tract

The absorption of copper from the ruminant digestive system is complex and difficult to confirm with research as there are many factors which can influence the uptake of copper from the digestive system and transport it to the blood stream (Gooneratne et al., 1989a).

Grace (1975) used sheep which were fitted with re-entrant cannulas at the proximal duodenum and terminal ileum to investigate where the copper introduced into the diet was being absorbed. The data from this trial showed that copper concentration in the digesta was relatively unchanged from the duodenum to the ileum but was significantly

less in the faeces. Therefore it was concluded that the only significant absorption of copper, by pasture fed sheep, occurred further down the digestive tract than the ileum, most probably in the large intestine (Grace, 1975). However Turner et al., (1987) injected copper solutions directly into each of the digestive compartments of sheep and the blood from around each digestive organ was collected and analysed for copper concentration. This showed that copper was absorbed in the abomasum and the small and large intestinal system. It is possible that excretion of copper in the bile confounded the results gained by Grace (1975) and that copper is in fact absorbed from much of the alimentary tract.

There are two types of mechanisms of absorption of metals through the intestinal wall, passive absorption and active absorption (Ashmead et al., 1985). Passive absorption is where the metal moves through the intestinal wall down a concentration gradient. Active absorption is where the metal is actively transported through the intestinal wall.

Some believe that active absorption only occurs in metals bound in an organic compound (Ashmead et al., 1985). However it is thought that the active transport mechanism may be used as a way to regulate metal absorption, for example it does not operate when copper concentration in the blood is high, therefore preventing copper toxicity. But if this theory is correct then it means that the active transport mechanism absorbs both free and organic metals. There is still a lot of debate in the area of metal absorption and until more research in the area is done many questions will remain unanswered.

The passive absorption of copper from the digestive tract into the blood stream is more complicated than simple diffusion. It is suggested that copper absorption is a two-stage process, where copper is absorbed from the lumen to the mucosal cell, where copper binds to binding sites in the wall of the digestive tract, and from here a specific mechanism operates transferring copper from the mucosal cell to the blood (Turner et al., 1987). The discovery of metallothionine, a metal-binding protein, in the mucosal cell has led to the suggestion that metallothionine may be important in the specific mechanism which operates to transfer copper from the mucosal cell to the blood (Turner et al., 1987).

Factors which influence the absorption of copper

Diet

The amount of copper absorbed from different forages can vary considerably (table 2). There is a dramatic difference in copper absorption in the ruminant between grazed herbage, brassicas, and cereals (table 2). Grazed herbage is estimated to have the lowest rate of copper absorption in the winter but this can differ considerably with fertilizer application, soil type, and climate. Both the brassicas and cereals are estimated to have a high copper absorption compared to silage, and grazed herbage.

Table 2. Estimates on the percentage of copper absorbed by Scottish Blackface ewes from seven major forage types (Underwood & Suttle, 1999).

Diet	Copper Absorption (%)	No. estimates
Grazed herbage (July)	2.5 ± 1.09	7
Grazed herbage (Sept./Oct)	1.4 ± 0.86	6
Silage	4.9 ± 3.2	7
Hay	7.3 ± 1.8	5
Root brassicas	6.7 ± 0.9	2
Cereals	9.1 ± 0.97	3
Leafy brassicas	12.8 ± 3.2	5

Organic matter

The composition of the diet influences copper intake and absorption by the amount of copper it contains, the availability of that copper, and the organic component of the feed (Suttle, 1991). A high level of organic matter in the diet provides free coppers ions in the rumen with many organic molecules to bind with it. These organic molecules bind the free copper so well that much of the bound copper is unable to be absorbed in the small intestine (Ashmead, 1993). Thus organic matter lowers the absorptive availability of copper from the diet.

Pastures have high levels of organic matter whereas cereals and brassicas contain low levels of organic matter. Therefore although pasture generally contains a higher copper concentration much of that copper can be unavailable to the animal.

Rumen and upper intestinal pH

The pH in the rumen as a result of diet also influences copper absorption and availability in ruminants (Suttle, 1991). A more acidic rumen environment promotes enhanced copper availability by an increased breakdown of thiomolybdates in the rumen and increased sulphide absorption from the rumen (Suttle, 1991). This means that because of a lower pH fewer thiomolybdates are formed in the rumen which allows more free copper to enter the abomasum and intestinal system. Therefore diets which promote a more acidic rumen pH, such as silage or cereals, will promote greater copper absorption due to them being rich in readily fermentable carbohydrates (Suttle, 1991).

Mineral interactions and antagonisms affecting copper absorption

Copper absorption is influenced to the greatest degree in the rumen, where antagonisms occur reducing the absorptive availability of copper in the intestinal system (Suttle, 1974). When the rumen is by-passed (by the reticulo-omasal orifice), meaning the copper no longer enters the rumen, the absorptive efficiency of copper in the lamb rises from 3.7% to 21.4% (Suttle, 1974). The most common antagonistic mineral to copper absorption is molybdenum, although sulphur, iron, and zinc have also been found to negatively influence absorption and utilisation of copper. Interfering factors, or antagonists, can act in two areas; absorption of copper from the digestive system or systemic utilisation of copper.

Molybdenum in the presence of sulphur

Ruminants have been found to develop signs of copper deficiency in instances where the copper status of the pasture is high (>10ppm) (Suttle, 1974). This is caused by high levels of copper antagonists being ingested in the diet. The minerals which antagonise the uptake of copper are molybdenum, sulphur, and iron (table 3) (Suttle, 1974).

Molybdenum and sulphur antagonise copper through the formation of thiomolybdates in the rumen. Thiomolybdates are compounds made up of a complex of copper, molybdenum and sulphur (Lee & Grace, 1997; Mason et al., 1988). Once copper is bound in the thiomolybdate compound it is unavailable for absorption in the small intestine therefore the bound copper passes out of the animal in the faeces (Lee & Grace, 1997). Because of this mechanism high levels of molybdenum, in the presence of sulphur, reduce the bioavailability of any copper ingested by livestock (table 3).

There are four main types of thiomolybdates which form; mono-thiomolybdates (MoS), di-thiomolybdates (MoS_2), tri-thiomolybdates (MoS_3), and tetra-thiomolybdates (MoS_4) (Lee & Grace, 1997).

Thiomolybdates are sometimes absorbed into the blood through the intestinal wall. Therefore thiomolybdates can act systemically to reduce copper availability further (by binding more free copper). These systemic thiomolybdates are eventually filtered out by the kidney and are excreted in the urine (Suttle, 1991).

Table 3. The effects of high iron (Fe) and molybdenum (Mo) levels on cattle liver and plasma copper levels and the effects on cattle reproductive performance (Phillippo et al., 1987).

Diet	Control	Fe Added	Mo Added
Cu (mg/kg DM)	4	4	4
Fe (mg/kg DM)	100	**600**	100
Mo (mg/kg DM)	0.1	0.1	**5**
S (g/kg DM)	2.8	2.8	2.8
Liver Cu (mg/kg DM)	59 ±5.4	5.9 ±0.5	4.5 ±0.7
Plasma Cu (mg/L)	0.80 ±0.02	0.13 ±0.02	0.12 ±0.02
Age at puberty (d)	285 ±9	299 ±7	343 ±1
Weight at puberty (kg)	313 ±11	310 ±11	283 ±6
Number pregnant	9 of 14	8 of 14	6 of 18

When higher thiomolybdates, tri-thiomolybdates and tetra-thiomolybdates, are absorbed through the intestinal wall into the blood they react with albumin causing copper to be bound by the albumin at a site other than the N-terminal locus which is where copper is normally bound to albumin (Suttle, 1991). This complex; containing thiomolybdate, copper and albumin, is so strongly bound that copper delivery to the liver is reduced (Mason et al., 1988; Suttle, 1991). The formation of thiomolybdates in the blood is shown by measuring the tri-chloroacetic acid insoluble (TCA-insoluble) copper fraction in the blood, this is the proportion of copper in the blood which is bound to thiomolybdates and unavailable to the animal for utilisation (Mason et al., 1988). A high TCA-insoluble measurement means that a large proportion of the copper in the blood is unavailable to the liver or the tissues, meaning the level of formation between thiomolybdates and albumin is high. Thus systemic thiomolybdates have a large impact on the amount of copper in the blood available for use by livestock.

Thiomolybdates circulating in the blood influence copper storage in livestock by its effects on the liver and biliary system (Suttle, 1991). Thiomolybdates act on the entero-

hepatic circulation in the liver causing copper to be removed from the liver via the biliary system (Suttle, 1991). This process occurs very quickly and efficiently. Sheep have been saved from chronic copper toxicity when given an intravenous injection of thiomolybdates (Suttle, 1991). The thiomolybdates clear the large amounts of copper from the liver, causing it to be excreted into the bile where it is then excreted in the faeces (Grace et al., 1996).

The formation of thiomolybdates is not thought to be the only mechanism which causes signs of copper deficiency in livestock. When molybdenum levels in the diet are high effects, such as poor fertility, which were once attributed to copper deficiency are now associated with toxic levels of molybdenum in the blood, called molybdenosis (table 3) (Howell & Gawthorne, 1987c; Phillippo et al., 1987). The effects of molybdenum toxicity, rather than copper deficiency, are often seen when the animal has not been exposed to high molybdenum levels for a long period as it takes a few weeks or more for molybdenum to cause a copper deficiency where high molybdenum levels in the diet will cause a increase in blood molybdenum levels in short space of time.

High molybdenum levels in the feed is effective in both alleviating copper toxicity, and causing copper deficiency (Suttle, 1974). Molybdenum continues to remove copper from the body in all situations, however the copper status of the animal determines whether it will have normal copper levels in the presence of high molybdenum or be deficient in copper (Cunningham, 1954). If the animal has toxic levels of copper, molybdenum supplementation will help reduce its toxic copper status. However if the animal has a normal copper status molybdenum will in most cases induce a copper deficiency. In New Zealand high molybdenum concentration in the feed usually causes a copper deficiency.

Sulphur

Sulphur alone has been found to reduce the absorptive availability of copper, through the formation of insoluble CuS; a molecule which renders the copper unavailable for absorption (Suttle et al., 1984). However when this experiment was repeated by (Grace et al., 1997) the findings showed that high sulphur, without the presence of high

molybdenum, did not reduce copper absorption. In soil ingestion in sheep it has been suggested by Suttle (1984) that an antagonism between sulphur, iron and copper occurs reducing the copper concentration in plasma (table 3). In this hypothesis iron and sulphur interact in the rumen forming either FeS or $FeSO_4$, and in the acidic environment of the abomasum these molecules are broken down, and the sulphur split off as a sulphide (S^{2-}) binds with copper (Cu^{2+}) forming the CuS molecule thus reducing the amount of available copper in the blood (Lee & Grace, 1997; Phillippo et al., 1987; Suttle, 1991).

Iron

High iron intake in livestock is associated with soil ingestion, high iron levels in the pasture, and high iron levels in the drinking water. Iron has been found to lower the copper status of both cattle and sheep (Suttle, 1991). It is thought that iron reduces the copper status of ruminants via its process of binding with sulphur in the rumen (forming FeS) (Suttle, 1991). The FeS molecule passes through into the abomasum where the gastric digestion breaks the molecule up releasing sulphur in the abomasum and intestinal system, this reduces the amount of sulphur available to form thiomolybdates in the rumen but increases the amount of free sulphur available in the intestinal system (Suttle, 1991). The free sulphur in the abomasum and intestinal system may bind to free copper forming CuS which reduces the free copper into an unavailable form reducing the amount of copper which is available for absorption by the animal (Suttle, 1991). Therefore the theory of process in which iron reduces copper is through the release of sulphur and subsequent increased concentration of sulphur in the abomasum and intestine system which may bind with free copper (Suttle, 1991).

The compound Fe_2O_3 can also inhibit copper absorption independent of sulphur (Suttle, 1991). Fe_2O_3 does this by absorbing copper making it unavailable for absorption by the animal (Lee & Grace, 1997).

Soil Ingestion

Soil ingestion has been found to reduce the copper status of livestock via antagonism of dietary copper with other minerals found in the soil such as molybdenum, sulphur, and iron (Suttle et al., 1984). Soil is ingested by grazing livestock when soil has contaminated the pasture due to pugging, when a high level of worm casts are present, or when a root crop is grazed (Grace et al., 1996). When pasture allowance is low, soil ingestion can make-up 10-14% of the dry matter intake in cattle and sheep, and in the winter months this percentage increases up to 40% in sheep (Grace et al., 1996). Suttle *et al.*, (1984) found that two of the three soils added to the diet of sheep in the presence of high sulphur levels caused a significant decrease in plasma copper concentration. These results showed that an antagonism was occurring between the three soils and copper causing a reduction in the amount of copper absorbed by the animal. Grace *et al.*, (1996) however found no difference in liver copper concentration between sheep given 100g of soil per day and sheep given no soil. Soil ingestion caused an increased in liver concentrations of B12 and selenium. It was concluded that soil may actually be beneficial as a source of minerals in the diet. Differing soil types may have contributed to the difference in findings.

Parasitism

Parasitism has been suggested as a factor causing copper deficiency in livestock. Adogwa *et al.*, (2005) studied 20 ewes over 12 weeks, half of which were infected with nematodes, and 5 ewes from each group were injected with copper. The results of this study showed that the introduction of nematodes reduced the serum copper and hemoglobin concentration of the animal (Adogwa et al., 2005). Despite being treated with injectable copper the infected group still had a lower copper and hemoglobin status than the non-infected group which had no copper supplementation (Adogwa et al., 2005).

Transport of copper

Systemic extra-cellular transport

Once copper has been absorbed from the lumen of the stomach and intestinal system of the ruminant it moves through the mucosal cells into the mesenteric blood and is transported to the liver and then to the rest of the body. Once copper has entered the blood it is bound to albumin, histidine or transcuprein (Sarkar, 2000).

Copper can be bound to many sites on the albumin protein, however the N-terminal region and cysteine residues are considered the primary transport sites (Harris, 2000; Sarkar, 2000).

Histidine is closely associated with albumin in the transport and release of copper, as histidine forms a complex with albumin and the copper bound to it stimulating albumin to release its bound copper as a 1:2 histidine-copper complex which allows cells to absorb the copper (Harris, 2000; Sarkar, 2000). Even though the histidine ligand never enters the cell it is thought to play a central role in the uptake of copper from the blood into the cell (Lee & Grace, 1997).

Ceruloplasmin performs the function of transporting copper, previously stored in the liver, via the blood to the organs and cells through-out the body (Gooneratne et al., 1989b). This protein is synthesized in the liver and excreted into the blood (Sarkar, 2000; Vulpe & Packman, 1995), and is the most abundant copper protein in blood; containing 70-95% of plasma copper (Harris, 2000; Vulpe & Packman, 1995). Each ceruloplasmin protein binds 6 or 7 copper atoms to a variety of binding sites on the protein (Sarkar, 2000). The copper bound to ceruloplasmin is bound as Cu^{2+} (Sarkar, 2000; Vulpe & Packman, 1995) and enters the cell without the protein in the form of Cu^{+} (Sarkar, 2000). Once the Cu^{2+} atoms have been released by ceruloplasmin at the cell they are reduced by a cell surface reductase to Cu^{+} so they can enter the cell via a transport protein (figure 3) (Gooneratne et al., 1989b; Harris, 2000; Sarkar, 2000).

However absorption of copper from ceruloplasmin into the liver involves the protein as well via the hepatolysosomal route, where ceruloplasmin containing the copper atoms is absorbed and separated from the copper atoms inside the liver (Gooneratne et al., 1989b). Once formed, ceruloplasmin has a half life of 37 hours in cattle, 70 hours in sheep, or 12 hours in the rat, therefore ceruloplasmin does not persist for long periods (Gooneratne et al., 1989a). Ceruloplasmin plays the most significant role in copper transport in the rat when compared to cattle and sheep, as it has been found as the only significant copper transport mechanism whereas in the cow it has been shown that 24% of the copper contained in her milk was derived directly from her diet rather than ceruloplasmin bound (Harris, 2000). This indicates that in ruminant's ceruloplasmin is not the only significant copper transporting factor.

Membrane Transport

This is the process where copper is transported from the apical surface of the cell into the cell. In yeast cells a gene called CRT1 has been found which specifically transports Cu^{+} not Cu^{2+} (Harris, 2000). Therefore the reductase which reduces Cu^{2+} down to Cu^{+} is essential in the cellular absorption of copper (Harris, 2000).

Intra-cellular transport

Elemental copper and copper proteins are found through-out the cell, in the nucleus, mitochondria, lysosomes, endoplasmic reticulum and cytosol (Harris, 2000; Sarkar, 2000; Vulpe & Packman, 1995). They are essential to the survival and functioning of the cell.

There are two main factors which are responsible for copper transport within the cell; glutathione and copper chaperones (Harris, 2000).

Glutathione, a ubiquitous cysteine-containing tripeptide, exists in millimolar quantities in the liver, brain, kidney, and many other cells (Harris, 2000; Sarkar, 2000; Vulpe & Packman, 1995). It binds Cu^{+} within the cell and transfers copper to the binding sites of

the cytosolic proteins; CuZnSOD, ceruloplasmin, hemocyanin, and metallothionein (Harris, 2000; Sarkar, 2000).

Copper chaperones are cytosolic peptides which form complexes with Cu^{+} and transport copper to specific proteins within the cell (Harris, 2000). Therefore copper chaperones perform a similar function to glutathione. Chaperones have target-specifying properties, where the copper is transferred to a specific area of the cell meaning delivery from chaperones is more uniform and controlled (Gooneratne et al., 1989a).

Copper storage

The liver has been well proven to be the primary storage organ of copper in the mammalian body (Bremner, 1987; Bremner & Mills, 1981). Copper is temporarily stored in the blood plasma also bound to protein such as ceruloplasmin and albumin. Once copper has been absorbed into the blood it is removed from the blood by the liver where it is stored, secreted into blood plasma, or excreted into the bile (Tao & Gitlin, 2003).

Liver Storage

Uptake of copper from the blood into the liver is performed by the hepatocytes contained in the liver, the CTR1 protein at the apical surface of the hepatocytes brings the copper into the cell where it is metabolised and released into the liver (Tao & Gitlin, 2003).

Once copper has been released from the hepatocytes it is transported with ceruloplasmin in the blood, excreted in the bile, or stored in the liver as metallothionein bound copper (Bremner, 1987).

Metallothionein is a protein which exists in the liver and many other tissues (Bremner, 1987). It is primarily involved in binding metals such as cadmium, zinc, and copper (Bremner, 1987). High zinc levels can negatively affect the storage of copper in the liver through competition for binding sites to metallothionine. However low levels of both copper and zinc increase the amount of metallothionein produced in the liver thus increasing the storage capacity of the liver increasing the amount of copper and zinc which can be stored (Bremner, 1987). High levels of copper in the blood has been found to stimulate increased metallothionein production in the liver allowing greater storage capacity in the liver (Bremner, 1987).

It has been shown that copper is released from the liver contained in the ceruloplasmin enzyme in the blood when blood copper concentrations are low, the exact mechanism for this process is unknown (Bremner, 1987; Bremner & Mills, 1981).

Metallothionein also helps maintain the homeostasis of copper as it stores more copper when copper concentration is high, and releases copper when blood concentrations are low.

Excretion of copper

There are three major pathways through which copper is excreted in ruminants; the faeces, the bile and urine. A large proportion of excreted copper is found in the faeces, which includes the copper excreted in the bile (Gooneratne et al., 1989b). Urine contains the least amount of copper but in the presence of thiomolybdates copper excretion through the urine increases dramatically (Gooneratne et al., 1989b).

Faecal copper excretion

Copper excreted via the faeces is a combination of copper from a variety of sources; the bile, saliva, gastric secretions, intestinal secretions, and un-absorbed dietary copper (Mason et al., 1988).

Only around 10% of dietary copper is absorbed from the diet of grazing livestock and much of the undigested copper is excreted in the faeces.

Biliary copper excretion

Bile copper excretion is regarded as the major pathway of copper excretion and much of the copper excreted in the bile ends up being excreted from the body in the faeces (Gooneratne et al., 1989b).

Copper excreted into the bile occurs via three routes; transbiliary, transhepatocellular, and hepatolysosomal (Gooneratne et al., 1989b). Transbiliary copper excretion is where copper is transported directly from the blood into the bile ductile and therefore it bypasses the hepatocyte (liver) (Gooneratne et al., 1989b). Transhepatocellular copper excretion is where copper is passed from the blood into the liver hepatocyte cells and from there is excreted into the bile (Gooneratne et al., 1989b). Hepatolysosomal copper excretion is where copper is taken up by the lysosomes, and is then excreted into the bile (Suttle, 1991).

Thiomolybdates can decrease the copper status of ruminants via its effect on the biliary excretion of copper (Gooneratne et al., 1989b).

Urinary copper excretion

Urinary copper excretion is considered to be a minor pathway for copper excretion in livestock which have a good copper status. However, in animals which are copper deficient urinary copper excretion can account for 25% of the unavoidable copper lost (Gooneratne et al., 1989b). The major pathway of copper lost via the urine is the blood as it is filtered by the kidney. Antagonists, namely tetra-thiomolybdate, have been found to increase copper excretion in the urine (Gooneratne et al., 1989b).

CLINICAL SIGNS OF COPPER DEFICIENCY IN RUMINANTS

Clinical manifestations of copper deficiency in livestock are closely linked to the enzymes in which copper is incorporated. Therefore clinical manifestations of copper deficiency are very broad, often affecting different species in different ways.

Clinical manifestations of copper deficiency in livestock were first reported in the 1940's, where copper responsive disorders such as enzootic ataxia and osteoporosis of lambs, and peat scours in cattle were found (Phillippo, 1983).

Unlike many other trace elements, such as cobalt and selenium, there are very few dose response and production response trials in copper deficient livestock. In addition much of the research in the area of copper and livestock has been performed on sheep and extrapolated to cattle. Thus the literature on the effects of copper deficiency on the health and production of cattle is not precise and is often open to debate.

Live-weight response

There is a limited amount of data showing a link between live-weight gain in cattle and copper supplementation and the data that is available does not show a clear relationship between the two. Studies have found both positive and negative effects on live-weight gain in livestock supplemented with copper (table 5). The research is further complicated by the addition of molybdenum, with it being unclear as to whether a drop in live-weight gain was caused by a copper deficiency or by high molybdenum concentration.

In most of the trials which achieved a positive live-weight response to copper, molybdenum was also supplemented at a relatively high concentration. Therefore, if molybdenum concentration in the feed is high you may get a positive live-weight response to copper supplementation (Ellison, 1992; Phillippo, 1983).

Live-weight response reference ranges, based on overseas data, in cattle show that no live-weight response to copper supplementation can be expected if the serum copper concentrations are above 8.0μmol/L and if liver copper concentrations are above 95μmol/kg FW (table 4).

Table 4. Growth response in cattle to copper supplementation when compared to mean serum and liver copper levels derived from overseas studies (Ellison, 1992).

Mean serum Cu (μmol/L)	**Growth response** (kg/day)
<1.5	0.33
1.5 - 5.5	0.18
5.6 - 7.9	0.08
>8.0	0
Mean liver Cu (μmol/L)	**Growth response** (kg/day)
<45	0.23
45 - 95	0.05
>95	0

Neonatal Ataxia

Neonatal ataxia or "swayback", as its commonly called in New Zealand, is a condition sometimes seen in New Zealand lambs from copper depleted flocks (McDowell, 2003). Swayback is a nervous disorder which causes the un-coordinated movement of the hind quarters, hence the name "swayback".

Swayback is caused by the demyelination of the central nervous system as a result of copper deficiency during growth and development of the feotus (Wilson & Grace, 2001). This condition occurs in lambs whose mothers are deficient in copper, thus the copper deficiency is present in the lamb long before it is born. Swayback has also been observed in lactating hinds which were subjected to an induced copper deficiency (Wilson & Grace, 2001). However it is not clear if this condition seen in adult hinds is related to the swayback seen in lambs.

Table 5. The effect of dietary copper and molybdenum concentration on live-weight gain in cattle (adapted from Phillippo 1983).

Author	Cu (mg/kg)	Mo (mg/kg)	Effect of Cu on live-weight gain
Bingley & Anderson (1972)	4.0-6.7	2.1-9.2	Positive
Jamieson & Allcroft (1950)	7.7-20.8	3.2-19.5	Positive
Field (1957a & b)	9.3-14	6.3-10.3	Positive
Miltimore *et al.*,(1964)	10.2	8.8	Positive
Thornton *et al.*, (1972b)	6.9-10.2	5.6-6.5	Positive
Poole *et al.*, (1974)	9.2	6.3	Positive
Morgan *et al.*, (1962)	6.7-11.5	3.4-6.7	Positive
Pierce *et al.*, (1976)	5.9-14.3	0	Positive
Smith & Thomson (1978)	2.5-5.5	3.5	Equivocal
Rogers & Poole (1977)	13.9	7.6	Positive
	13.4	4.9	Negative
McPherson *et al.*, (1979)	4.5-12.5	0.7-1.8	Equivocal
McPherson & Dixon (1980)	4.4-7.0	0.8-1.5	Equivocal
Donaldson (1960)	5.5	0	Equivocal
Suttle *et al.*, (1980)	3.5-9.6	0.7-1.3	Negative
Todd *et al.*, (1967)	5.0-7.6	1.0-2.3	Negative
Phillippo *et al.*, (unpublished)	3.2-5.6	0.7-1.6	Negative
Engel *et al.*,(1964)	4.9	0	Negative
Hidiroglou & Jenkins (1975)	5.0-11.0	0	Negative
Haag & Adams (1958)			Negative
Gartner *et al.*, (1968)			Negative
Stoszek (1976)	4.6-6.6	1.2-2.4	Negative
Gurnett & Lawrence (1978)			Negative

Bone disorders

In livestock un-even bone growth and weak bones as a result of copper deficiency generally occur in growing animals (Audige et al., 1995; Underwood & Suttle, 1999).

Severely copper deficient calves and lambs will exhibit swelling or enlargement of the ends of leg bones, and bones will break easily due to weakness (osteoporosis) (Underwood & Suttle, 1999). Beading at the center of the ribcage may also be seen in copper deficient calves and lambs due to the over growth of the costrochondral junctions (Audige et al., 1995).

In copper deficient red deer skeletal abnormalities have been observed (Audige et al., 1995). Clinical signs manifest as skeletal abnormalities such as swollen hocks, carpal joints, the outward rotation of the hind legs with hocks touching (figure 2), and lameness (Audige et al., 1995).

Figure 2. Severely copper deficient fawn shown skeletal abnormalities where the hocks are touching (McDowell, 2003).

Bone disorders in copper deficient ruminants are often accompanied by connective-tissue disorders (McDowell, 2003; Underwood & Suttle, 1999). Connective-tissue disorders are

generally displayed by the gait of the animal; where it may be pigeon-toed, stiff-legged, or appear to bunny-hop (Bruere, 1982; Underwood & Suttle, 1999).

This manifestation of copper deficiency is linked with the enzyme "Lysl oxidase" where the elastin and collagen formation is reduced in the animal forming poorly functioning connective-tissue and weak bones (Hunter, 1977).

Reproductive Performance

Studies show mixed results in the area of copper and fertility in ruminants, and it remains an area of contention between researchers. Phillippo et al., (1987) supplemented cattle with 400mg of copper glycinate which significantly improved fertility rates. Pregnancy rates where 9 of 14 (72%) for copper supplemented cows and 6 of 18 (53%) for non-supplemented cows (table 3). A dose-response trial was run on four farms, with mean plasma copper concentrations below 0.3mg/l. This trial showed that copper supplementation had no effect on conception percentage even though plasma copper levels increased (Hafez & Hafez, 2000). Therefore it is still not conclusively proven that copper supplementation improves conception rates in dairy cows on all farms.

Copper is important in the functioning of the reproductive system through its effects on the estrous cycle, ovarian activity and the synthesis and release of gonadotropins (Hafez & Hafez, 2000). The initiation of the estrous cycle and maintenance of pregnancy is controlled by the release of the hormones; estrogen, Luteinising hormone (LH) and follicle stimulating hormone (FSH) (Lonergan et al., 2003). Secretion of the hormones LH and FSH is controlled by gonadotropin-releasing hormone (GnRH) (Underwood & Suttle, 1999). Copper is also thought to be important in the synthesis of GnRH from the hypothalamus increasing its concentration and through this mechanism it is thought that copper may have an effect on the release of gonadotropins (McDowell, 2003). Therefore, copper deficiency may cause reduced conception rates, anestrous or fetal resorption in livestock (Singh et al., 1998).

Copper dependant superoxide dismutase also has some function in reproduction in the dairy cow; providing protection to the developing oocyte during early pregnancy against oxidative damage (Underwood & Suttle, 1999).

Scouring and Diarrhoea

This condition is more noticeable in cattle than sheep as the excreta is generally more fluid (Underwood & Suttle, 1999). Diarrhoea in cattle is caused by high molybdenum levels in the blood, however it is also the molybdenum excess which causes a copper deficiency hence why peat scours is often associated with a copper deficiency (Mills et al., 1976). Cattle with severe diarrhoea will respond positively to copper treatment within 12 hours (McDowell, 2003; Underwood & Suttle, 1999). Mills (1976) found that in cattle with an induced copper deficiency with severe diarrhoea the administration of sulphadimidine-streptomycin-kaolin (a compound known to remediate diarrhoea in cattle) did not reduce the severity or incidence of diarrhoea in cattle, but when a single oral dose of 10mg of copper sulphate was given the diarrhoea ceased within 12 hours.

It is still unclear however as to the mechanism which remedies diarrhoea when copper is dosed. Whether it is the increase in copper available to the animal for utilisation or the effect of putting more copper into the rumen allowing more thiomolybdates to be formed reducing the amount of molybdenum available to the animal thus reducing the effects of molybdenum toxicity.

Hair depigmentation and defective keratinisation

Fading hair colour, due to a lack of pigment, is commonly seen in cattle which are copper deficient. The hair appears dull in colour; black hair turns grey or brown, and is often associated with thick and un-tidy hair in spring due to the delayed loss of the winter coat (Underwood & Suttle, 1999). In cattle the effects of depigmentation are especially apparent around the eyes (McDowell, 2003). In sheep depigmentation shows most prominently in the wool of black sheep which turn grey or lighter in colour.

In livestock defective keratinisation of wool/hair is another commonly seen manifestation of copper deficiency where the production of the copper dependant enzyme tyrosinase is reduced impacting the pigmentation of hair and wool. In sheep defective keratinisation is more prominent causing the animal to produce straighter hair-like wool, often referred to as steely wool, most readily seen in fine wool breeds such as Merinos (Underwood & Suttle, 1999).

Anaemia

Anaemia has been observed in livestock which are copper deficient. Prolonged copper supplementation in livestock reduces the effects of anemia, thus showing its link to copper (Ellison et al., 1986). Ceruloplasmin is the copper dependant enzyme which is essential in the transport of iron throughout the body and iron is needed for erythropoiesis (the creation of red blood cells). Therefore, a copper deficiency reduces the amount of ceruloplasmin produced thus reducing the effectiveness of the iron transport system leading to anemia due to a lack of iron. Blood copper concentrations of 1.5-3.0 μmol/L in cattle or sheep are associated with signs of anaemia. However, where copper status is this low many other clinical copper deficiency signs will also be displayed (Underwood & Suttle, 1999).

The first report linking copper deficiency to erthropoiesis was in 1931 where a jersey heifer had a haemoglobin concentration of 5.9g/100ml of blood. After supplementation with copper the animal had a haemoglobin concentration of 13.7g/100ml of blood (Underwood & Suttle, 1999).

In New Zealand the incidence of anaemia in livestock has commonly been associated with Heinz-body anaemia or post-parturient haemoglobinuria (McDowell, 2003; Smith & Coup, 1973), where Heinz-bodies develop in the red blood cells of grazing livestock. The development of Heinz-bodies is a by-product of poor erthropoiesis function and may be used as an indicator of anemia in livestock (Smith & Coup, 1973). Suttle & Jones (1987) showed a reduction in Heinz-body concentration in the blood of lambs as a result of

copper supplementation showing a strong link between copper status and erthropoiesis function.

Cardiovascular disorders

Cardiovascular disorders can be associated with copper deficiency in livestock, known as "falling disease". But this disorder is rarely seen in cattle (Underwood & Suttle, 1999). Falling disease occurs where the animal will die suddenly when put under stress, exertion, or even excitement. The enzyme lysl oxidase is important in the formation of the collagen in the vessels of the heart giving them strength.

Post-mortem examination of falling disease victims often reveals the rupture of major vessels and small lesions of the heart (Wikse et al., 1992), lesions are caused by slow and progressive degeneration of the myocardium, which is replaced by fibrotic tissue and an iron accumulation (Wikse et al., 1992).

DIAGNOSIS OF COPPER DEFICIENCY IN RUMINANTS

The diagnosis of copper deficiency is a function of looking for both the clinical manifestations of copper deficiency in ruminants and the analysis of tissues of the animal which indicate its copper status. This section will cover the analytical part of diagnosis.

Assessing the copper status of livestock is important, not only to confirm the presence of deficiency but also to predict whether supplementation may be required to prevent production loss.

The laboratory techniques used for this analysis are very important in terms of consistency of results and methods used for analysis.

Table 6. The tissue reference ranges used to diagnose copper deficiency in cattle, sheep and deer (Grace, personal communication, 2008).

	Deficient	Marginal	Adequate
Serum Cu (μmol/L)			
Cattle	<4.5	4.5 - 8	>8
Sheep	<4.5	4.5 - 8	>8
Deer	<5	5 - 8	>8
Liver Cu (μmol/L fresh tissue)			
Cattle	<45	45 - 95	>95
Sheep	<45	45 - 95	>95
Deer	<60	60 - 100	>100
Serum Ferrioxdase (IU/L)			
Cattle	<7	7 - 14	>14

Tissue reference ranges

A reference range is generally made up of three numbers or ranges; deficient, marginal, and sufficient (or adequate) (table 6). When biochemical data such as blood or liver copper concentrations are collected on a herd the mean of these values can be compared with the published reference ranges to see if the herd has a deficient, marginal or sufficient copper status. This then assists decisions on whether or not the herd needs to be supplemented with copper.

To publish a reliable reference range a significant amount of research must be done on the clinical effects of deficiency and the response to supplementation in the situation where deficiency arises. Biochemical data from various overseas studies on the response of livestock to copper treatment have been collected by researchers such as (MacPherson & Gray, 1985; Suttle & Angus, 1978; Suttle et al., 1972; Wilson & Grace, 2001). This research provides the standard for what is deficient, marginal, and sufficient for the biochemical test being looked at.

Tissue copper

The analysis of various animal tissues is performed in an effort to find the copper concentration contained in those tissues thus giving an indication of the copper status of the animal. The tissues commonly analysed are; liver and blood (whole blood, plasma, or serum) (Dewes et al., 1990). However other tissues, such as; hair, blood for ceruloplasmin activity, and blood for erythrocyte superoxide dismutase activity can also be analysed in most laboratories (Kincaid, 1999).

Liver is the most reliable tissue for assessing copper status (Underwood & Suttle, 1999). This is because of the characteristics of the storage and movement of copper throughout the body. The liver is the point where the largest proportion of copper is stored in the body, whereas the blood is the primary carrier medium of copper to and from the liver and throughout the body. Therefore liver analysis gives a good indication of the animal's store of copper whereas analysis of the blood only gives an indication of the amount of

copper which has been absorbed from the diet or released from the liver into the blood at that point in time from the liver (figure 3). However, once the liver reaches a critical low point, the blood values will also be low in copper (table 6) (Mills, 1987). When this occurs blood can be used as an indicator that copper supplementation is required (Laven et al., 2007). However, at no other point is blood copper concentration very useful as an indicator of the copper status of the animal.

Liver samples are either taken via liver biopsy on live cows or taken from the livers of cull cows which have been sent in for slaughter. Most laboratories analyse the copper concentration in tissue (liver and blood) samples using Atomic Absorption Spectrophotometry (AAS), the method for this technique is described in the methodology section of this thesis. The accuracy of this method of analysis is ±100 μmol/kg fresh weight (FW) in a liver containing 2400 μmol/kg fresh weight of copper (NZVP, personal communication).

Blood copper analysis is often used for measuring the status of livestock as it is relatively in-expensive and easy to collect. However, veterinarians and farmers are realising the shortfalls of blood analysis for copper status determination and moving to liver analysis as it is more reliable and useful for longer term assessment of requirements. The interpretation of blood copper analysis in the determination of livestock copper status is further confused by pathological changes in the animal as these cause changes in blood copper concentrations (Ellison, 1992).

Three blood factors are commonly used for copper analysis; whole blood, serum, and plasma. In New Zealand total copper in the whole blood, serum, or plasma is commonly measured. Within the blood ceruloplasmin concentration is often measured as an indicator of copper status. This test is commonly called a blood ferroxidase test (Claypool et al., 1975).

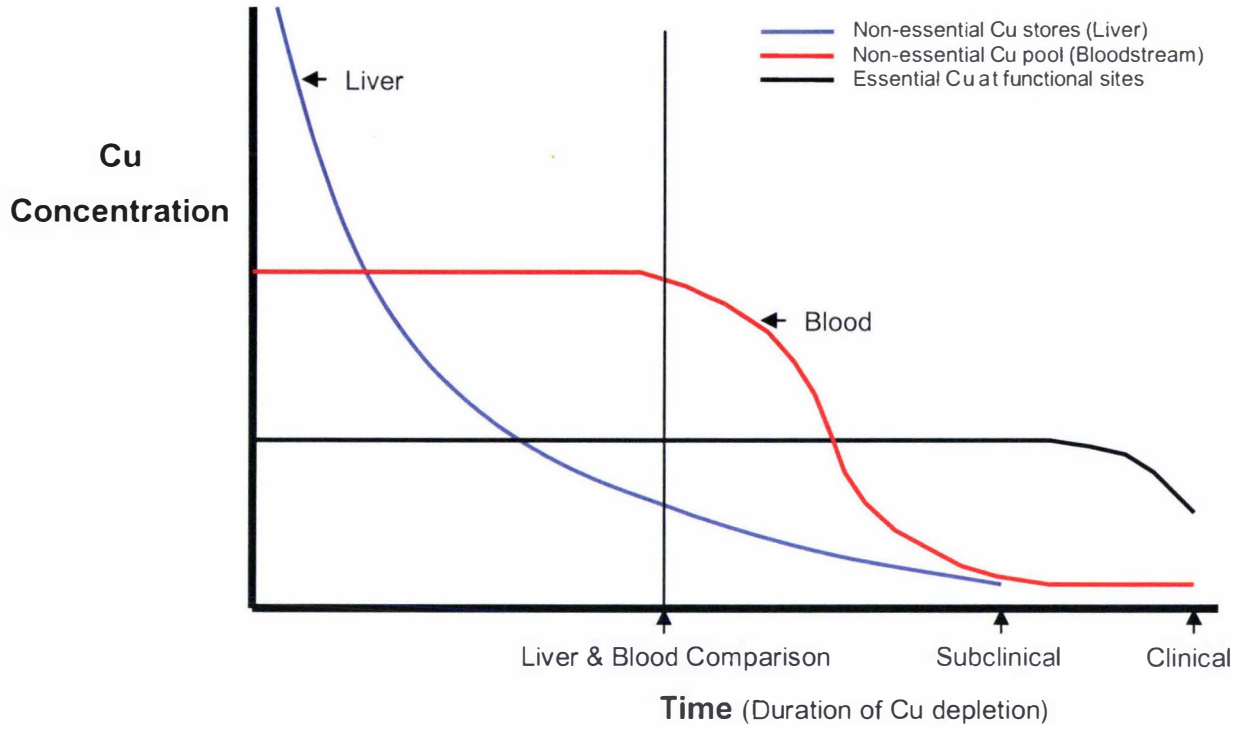


Figure 3. Graph of the changes in copper status in the liver, blood, and sites requiring copper for function over a period of copper depletion, and the point where sub-clinical and clinical copper deficiency occurs (Minatel & Carfagnini, 2002).

Copper concentration and ceruloplasmin activity is generally significantly lower in blood serum than in blood plasma, as a proportion of copper is sequested in the blood plasma during blood clotting (Mills, 1987). Laven et al., (2007) found that from 125 cattle the serum copper fraction was 2.92μmol/L less than the plasma copper fraction, indicating that a significant portion of the copper moves into blood plasma during clotting.

West & Vermunt (1994) found that a significant portion of cattle with low blood copper concentrations had adequate liver copper concentrations and indicating that blood copper concentration cannot be solely relied upon to diagnose copper deficiency (figure 4).

Other methods of copper status determination in livestock

The enzyme super oxide dismutase has been suggested as a reliable indicator of copper status in livestock (Legleiter & Spears, 2008). Super oxide dismutase appears to react more slowly than serum copper to rises and falls in copper levels and therefore is more stable. However, it is unknown if it is affected by pathological influence (Ellison, 1992). This method of determination of copper status is not commonly used in New Zealand as it has not been well researched.

Although biochemical analysis of copper dependant enzymes may be more precise in defining copper deficiency; it is less useful for deciding when the supplementation of copper is required to avoid production loss. Liver copper is considered the most useful predictor for assessing the need for copper supplementation in livestock.

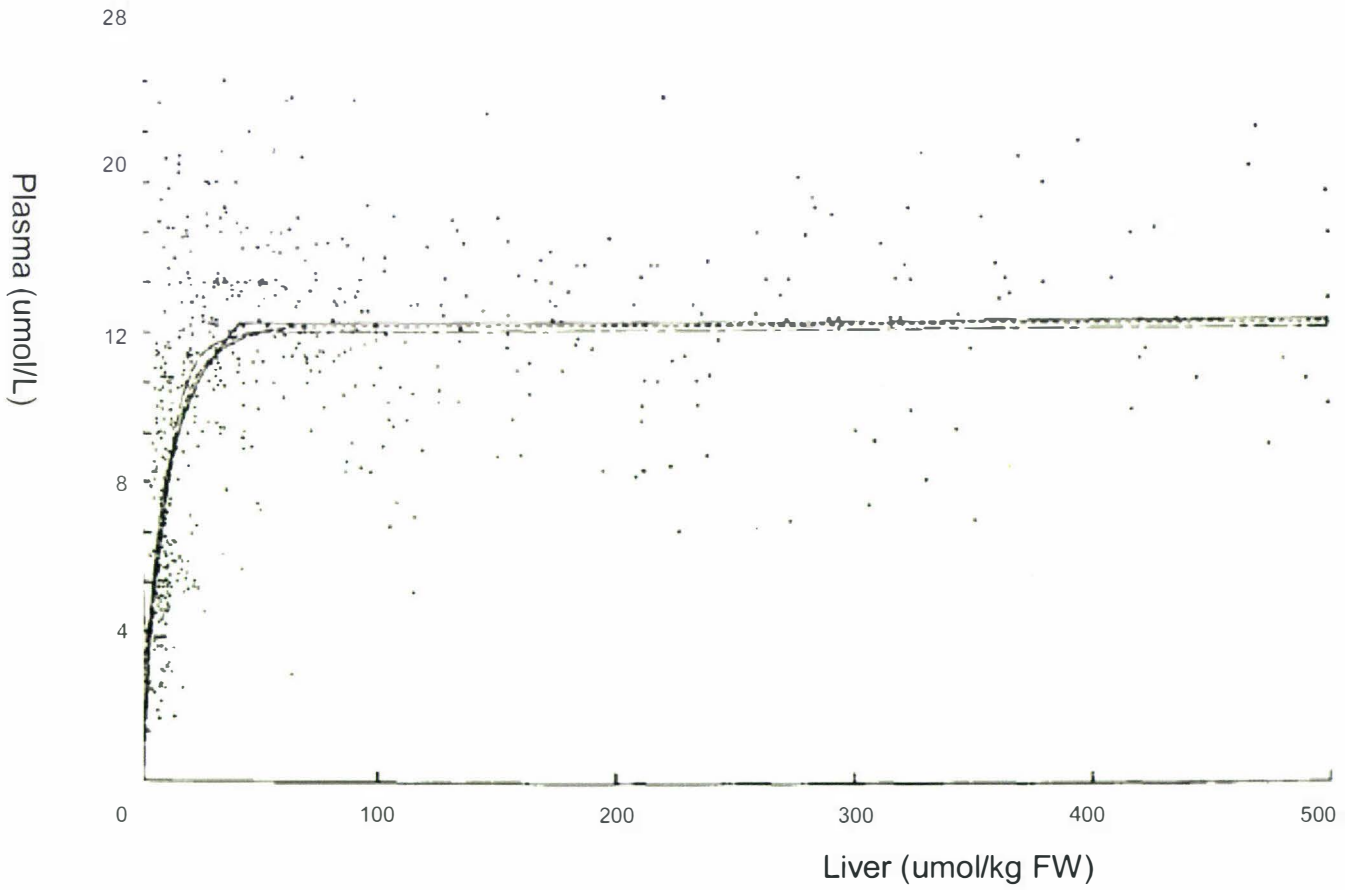


Figure 4. Diagram showing the correlation between liver copper stores (umol/kg FW) and blood plasma copper concentrations (µmol/L) in cattle (Claypool et al., 1975).

Plasma diamine oxidase activity has also been proposed as a measure of copper status. Diamine oxidase is a copper containing enzyme responsible for the oxidation deamination of diamines, their derivatives, and histamine (O'Connor, 1992). Legleiter & Spears (2008) found that diamine oxidase levels correlate well with other indices of copper status (73% to 87%). This shows that diamine oxidase levels may be a useful tool in determining the copper status of livestock (Grace, 1994).

Copper intake

The diet of New Zealand dairy cows generally consists of pasture and supplements such as hay, maize silage, grass silage, and concentrate rations. Beef cattle and sheep usually receive a diet of pasture and hay only.

Measuring the intake of the animal and the nutrient status of the forage is a useful tool in predicting and managing copper deficiencies in livestock. However forage measurements must be complemented with animal tissue measurements for accurate decisions to be made.

It is important not to allow contamination of samples, with contaminates such as soil, urine, or faeces which can cause erroneous results from the lab. A full range of trace elements can be measured, including many antagonistic trace elements which reduce copper status in livestock such as molybdenum, iron, and sulphur.

COPPER REQUIREMENTS FOR CATTLE

Nutrient requirements are calculated based on the daily nutrient intake needed to ensure good health and optimum performance (table 7) (Grace, 2004). These requirements are based on various dose response trials performed for each of the nutrients where cattle of various body weight and milk production are fed various levels of nutrients and the response is measured.

The copper requirement of cattle, and the amount of dietary copper needed to fulfill that requirement, is a complex subject as it is dependant not only on the animal, body weight and milk production, but also on the diet. Antagonists, such as molybdenum, zinc, iron and sulphur, all increase the amount of copper the cow requires, for example increasing pasture molybdenum from 1 to 3 mg/kg DM decreases the absorbable copper from 5 to 2.5% (Grace, 2004). In this situation the cow's dietary requirement for copper would double. The presence of these antagonists is factored into the copper requirement as the coefficient of absorption, where if high levels of antagonists are present the coefficient of absorption would be high causing the calculated copper requirement to also be higher to account for the high coefficient of absorption.

To accurately predict the copper requirement of cattle, taking into account antagonists, both feeding studies and a quantitative factorial model (figure 5) approach is used.

Quantitative factorial model

The factorial model gives the nutrient requirement for the animal based on the amount of nutrient which is used and lost from the body. In cattle the factorial model for copper measures endogenous loss of copper, the copper requirement per kg of weight gain, the amount of copper which the developing feotus requires and the copper requirement for milk production (figure 5) (ARC, 1980). The endogenous loss of copper is the most difficult to summarise as it varies significantly based on the diet and the level of antagonists in the diet, namely molybdenum.

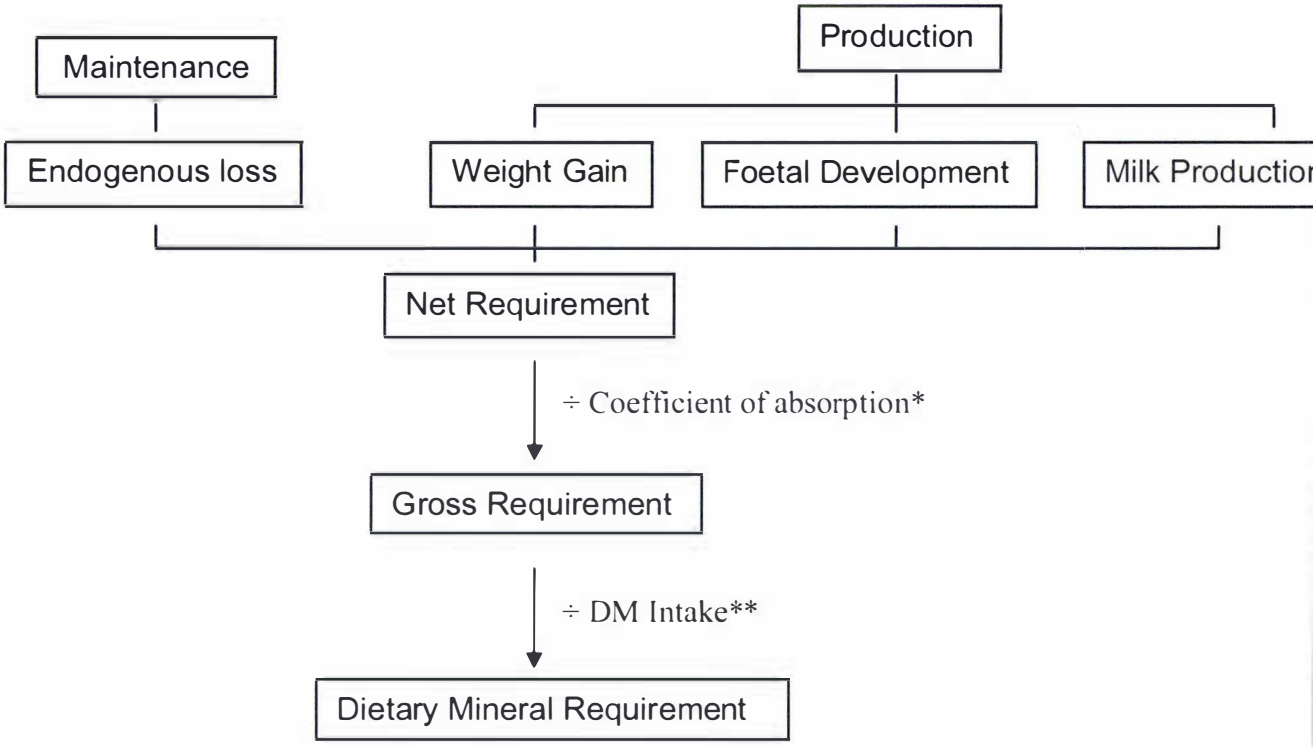


Figure 5. Factorial model for the factors involved in the determination of copper (mg) requirements for dairy cows. (*where molybdenum concentration is 1 mg/kg DM), (**where DM intake is 17 kg). (ARC, 1980; Grace, 2004).

Feeding studies

Feeding studies are research projects which are conducted in order to establish the nutrient requirements of a specific animal. These studies have been conducted on dairy cattle of various live-weights and milk production to establish the true copper requirement of dairy cattle. For example Xin et al. (1993) found that where non-lactating pregnant cows where fed 5.5mg copper/kg DM, liver copper concentration declined steadily until parturition. However when they were fed 10mg copper/kg DM liver copper concentration was maintained (Xin et al., 1993). This showed that the dietary copper requirement for dry dairy cows appeared to be around 10mg copper/kg DM.

When feeding studies on a specific nutrient have been repeated the requirements for each animal can be summarised (table 7).

Table 7. Dietary copper requirements for dairy cattle (ARC, 1980).

	Liveweight (kg)	Weight gain, stage of pregnancy or milk yield	Dietary Requirement (mg/kg DM)
Pre-ruminant	100	0.5 kg/day	7.1
		1.0 kg/day	7.6
	200	0.5 kg/day	6.8
		1.0 kg/day	6.5
	300	0.5 kg/day	6.8
		1.0 kg/day	5.3
Dairy Cow			
maintenance	500		10
late pregnancy	500	9 months pregnant	10
lactation	500	10 kg milk/day	11
	500	20 kg milk/day	9.4
	500	30 kg milk/day	9.1

SUPPLEMENTATION OF COPPER IN RUMINANTS AND THE DIFFERENT FORMS OF COPPER AVAILABLE

The form of copper supplementation used to prevent deficiency varies considerably depending on the degree of deficiency, the facilities available on the farm (such as the water system) the type of stock being farmed (dairy or beef) and the level of interfering factors such as molybdenum.

Different forms of copper supplements

Oral supplements include salts, dry powders, soluble dry powders, and solutions. The most commonly used oral supplements are; copper sulphate, copper oxide, copper amino acid chelates, copper complexes, and copper proteinates. These oral supplements fit into two main categories; organic and inorganic copper supplements.

Organic copper supplements

Organic, or chelated, copper supplements where created in response to situations where livestock were being supplemented with inorganic copper but adequate copper status was not being achieved. This situation arose due to the relatively low bioavailability of inorganic copper supplements due to antagonism of the supplemented copper in the digestive tract. The idea that chelated minerals may be more efficiently absorbed in livestock was first identified by Neathery et al., (1972) where corn fertilized with radio labeled zinc was grown and fed to livestock. The livestock fed the corn had contained

40% more zinc in their blood than livestock supplemented with an inorganic zinc supplement instead of the corn. This led to the idea that the zinc chelated naturally by the plant may be absorbed more efficiently than the non-chelated inorganic zinc supplemented (Neathery et al., 1972).

The term "chelate" is derived from the Geek work chel'le' for pincer like claws (Kratzer & Vohra, 1986). This term describes how a mineral molecule is held by a chelating agent in a heterocyclic ring formation (Hartle & Ashmead, 2006). The chelating agents most commonly used to chelate minerals are amino acids, citric acid, picolinic acid, lactic acid, peptides, and other synthetic chelating agents (Kratzer & Vohra, 1986). A suitable chelating agent must be of low molecular weight (below 1000 Daltons preferably) and should have a high stability constant, meaning the bonds it forms are strong. Once a mineral has been chelated it has been incorporated into an organic compound, therefore the chelated mineral is termed an organic mineral.

The four main types of organic mineral supplements currently available in New Zealand are; amino acid chelates, amino acid complexes, proteinates, and polysaccharide complexes. All four are termed organic minerals as they are free metals bound to organic compounds; amino acids, partially hydrolysed proteins, or polysaccharides (Ashmead & Ashmead, 2004).

Both the amino acid chelate and complex are compounds made up of free metals bound to amino acids (Spears, 1996). It is thought that complexed minerals are held by weaker bonds than a true amino acid chelate. However there is a large amount of variation between different types of amino acid chelates and complexes (Ashmead & Samford, 2004). The Association of American Feed Control Officials states that both the amino acid complex and amino acid chelate are products resulting from the reaction of a mineral with amino acids but outlines that the average weight of the amino acid chelate must not exceed 800 Daltons and that the molar ratio must be between 1 and 3 amino acids to 1 mineral ion (Spears, 1996). Therefore the complex may have a molecular weight of less than 800 Daltons and have a molar ratio of 1:1, 2:1, or 3:1 but unless it is classified as fulfilling these criteria it cannot be termed a true amino acid chelate. Even in the face of this classification it is still unknown whether an amino acid chelate has a greater

bioavailability than a complex as the dearth of research in the area makes it very difficult to establish the practical differences between the two.

The proteinate is a compound made up of a free metal bound to partially hydrolysed proteins (Spears, 1996). Because the free metal is bound to partially hydrolysed proteins, rather than amino acids as is the case in the amino acid chelate and complex, the proteinate is large in size being anything from 10,000 to 100,000 Daltons in molecular weight. Where the general standard for an amino acid chelate is that is less than 1000 Daltons in size (Kratzer & Vohra, 1986).

Because organic minerals where first developed to combat mineral deficiencies, which were not being solved by supplementing with inorganic minerals, the amount of elemental mineral which is absorbed and utilised by the animal is important.

Chelated minerals are digested and absorbed by ruminants in a different way to non-organic minerals. It is well known that interactions between free copper and other free metals, such as molybdenum, occur in the digestive system (the rumen through to the duodenum). These interactions reduce the copper which is available for absorption and utilisation by the animal. The theory behind chelated (organic) copper is that it does not interact with other free minerals in the digestive system and therefore the supplemented copper does not form complexes which reduce the absorptive efficiency of the copper (Spears, 1996). Chelated copper is thought to pass through the digestive tract with a high percentage of it reaching the intestine intact and available for absorption into the blood. There are two specific modes of interactions between free copper which occur in the digestive system of the ruminant reducing its availability.

Firstly interaction occurs between free minerals in the rumen, omasum, abomasum, and intestinal lumen forming insoluble complexes which are unable to be absorbed and thus pass out of the animal in the faeces. An example of this process is the formation of thiomolybdates.

Secondly interaction between free metals occurs at the intestinal wall where free metals are absorbed through the intestinal wall and into the blood (Ashmead, 1993). A high

concentration of free irons in the digestive tract will reduce the amount of free copper absorbed by overloading the transport molecule transferring. This is the active transport molecule which transports both free irons through the intestinal wall into the blood (Ashmead, 1993). The different mechanisms of absorption from the intestine of free copper and organic copper are imperative to the difference in bioavailability between free and chelated copper. Free copper is transported both passively and actively through the intestinal wall. However there are several feedback loops where free copper is transported back out of the intestinal wall into the lumen where it is reabsorbed or is passed out of the animal undigested in its faeces (Kratzer & Vohra, 1986).

There is inconsistency in the results obtained from research in the area of organic copper supplements verses inorganic copper supplements (table 8). Most of the research performed on organic copper has been on the copper lysine complex verses inorganic copper such as copper sulphate or copper oxide. Most of these studies have shown no significant difference in copper absorption between the organic and inorganic copper sources (table 8). Research has been performed on other types of organic copper supplements such as the copper glycine complex and copper proteinate. Some of this research has shown significant differences between the organic and inorganic copper sources but as with all supplement based research careful attention must be paid to the planning of the trial to ensure it is sound research.

Sustained release oral supplements

These include copper oxide needle boluses, soluble glass boluses, and polymer sustained release boluses (Wakelin, 1992).

Copper oxide needle boluses are made up of needle shaped copper oxide particles, approximately 4-5mm long and 1mm thick, contained inside a gelatin capsule. The capsule quickly dissolves in the liquid environment of the rumen allowing the copper oxide particles to fall to the bottom of the rumen where they eventually pass through the reticulo- omasal orifice and lodge in the folds of the abomasum. The acidic environment of the abomasum slowly dissolves the needles over a period of a few weeks releasing the

copper making it available for absorption in the small intestine of the animal (Wakelin, 1992).

Soluble glass boluses are a phosphate-based soluble glass administered down the esophagus into the rumen where they lodge and begin to dissolve over a period of up to a few months (Wakelin, 1992). They are shorter acting than the copper oxide needles, however have been found to raise copper levels significantly.

A sustained release product known as "Alltrace" consists of an inactive core coated with trace elements then coated with a polymer resin (Wakelin, 1992). Once the bolus is administered the slightly acidic environment of the rumen dissolves the trace elements through the flat end of the bolus but due to the surface area available this process is restricted making it slow release (Ellison, 2002). These boluses are administered down the esophagus ending up in the reticulum or the rumen.

Parental copper supplements

There are three types of copper injections available; copper di-ethylamine oxyquinoline sulphonate (CuDOS), copper glycinate, and copper calcium edetate (Bohman et al., 1984; Wakelin, 1992). These injections provide a dose of copper subcutaneously which is released into the blood over a period of a few days or possibly weeks. From there it is transported by the blood into the liver where it is stored for use as it is needed over the following 2-3 months. The major differences between the different types of copper injections are the period for which they last, the amount which can be administered safely and the level of skin reaction which occurs at the time of administration.

The copper di-ethylamine oxyquinoline sulphonate injection is highly soluble; therefore it forms a water soluble complex with copper making it readily absorbable from the injection site. However because it is so readily absorbed it carries an increased risk of copper toxicity. Because of this risk it is not commonly used in the New Zealand agricultural industry and is not registered for use in sheep and cattle.

Table 8. Summary of the peer-reviewed data on the efficacy of chelated copper supplements in livestock.

Reference	Copper Source (Organic & Inorganic)	Animals (per group)	Molybdenum (mg/kg DM)	Significant Difference	Comments
Kincaid *et al.*, 1986	Proteinate[5] vs Sulphate	15 Calves	5	✓	Significantly higher plasma but not liver copper concentration.
Wittenberg, K.M., *et al.*, 1990	Proteinate[5] vs Sulphate	12 Steers	10	-	
Nockels, C.F., *et al.*, 1993	Complex[1] vs Sulphate	4 Calves	-	✓	Study was flawed as a greater level of complex was supplemented.
Ward *et al.*, 1993	Complex[1] vs Sulphate	21 Steers	5	-	
Kegley, E.B., *et al.*, 1994	Complex[1] vs Sulphate	5 Calves	-	-	
Du, Z., *et al.*, 1996	Proteinate[2] vs Sulphate	4 Cows	-	-	
Luo, X.G., *et al.*, 1996	Complex[1] vs Sulphate	12 Lambs	-	-	
Ward *et al.*, 1996 (exp 1)	Proteinate[4] vs Sulphate	6 Heifers	-	-	
Ward *et al.*, 1996 (exp 2)	Proteinate[4] vs Sulphate	10 Heifers	5	-	
Eckert, G.E., *et al.*, 1999	Proteinate[3] vs Sulphate	10 Ewes	-	✓	Group receiving copper sulphate had significantly greater liver copper.
Olson, P.A., 1999	Complex[1] vs Sulphate	80 Cows	-	-	
Rabiansky, P.A., *et al.*, 1999	Complex[1] vs Sulphate	8 Heifers	5	-	Individual groups were not meaned for liver copper concentration at the start of the study.
Engle, T.E., *et al.*, 2000	Proteinate[4] vs Sulphate	10 Steers	-	-	
Bailey, J.D., *et al.*, 2001	Complex[1] vs Sulphate	12 Heifers	10	✓	Group receiving the copper complex and sulphate mix had significantly higher liver copper.
Hatfield, P.G., *et al.*, 2001	Complex[1] vs Sulphate	6 Ewes	-	-	
Muehlenbein, E.L., *et al.*, 2001	Complex[1] vs Sulphate	75 Cows	-	-	Copper measurements only taken on day 10 and 30 post calving.
Dorton, K.L., *et al.*, 2002	Complex[1] vs Sulphate	10 Steers	-	✓	At 20mg/kg DM the complex achieved significantly greater liver copper concentration, at 10mg/kg DM there was no difference.
Yost, G.P., *et al.*, 2002	Complex[1] vs Sulphate	10 Heifers	15	-	
Arthington, J.D., *et al.*, 2003	Complex[1] vs Sulphate	8 Heifers	-	-	
Mullis, L.A., *et al.*, 2003	Proteinate[3] vs Sulphate	10 Steers	334[a]	-	
Ahola, J.K., *et al.*, 2004 (Yr 1)	Proteinate[2] vs Sulphate	59 Cows	-	✓	Groups receiving copper proteinate had significantly greater liver copper levels in yr 1 only.
Ahola, J.K., *et al.*, 2004 (Yr 2)	Proteinate[2] vs Sulphate	62 Cows	-	-	
Salyer, G.B., *et al.*, 2004	Polysaccharide Complex[8] vs Sulphate	54 Heifers	-	-	However the group supplemented with the sulphate had significantly greater ovalbumin titers than the group supplemented with the polysaccharide complex.

Reference	Copper Source (Organic & Inorganic)	Animals (per group)	Molybdenum (mg/kg DM)	Significant Difference	Comments
Nocek, J.E., *et al.,* 2006	Complex[1] vs Sulphate	143 Cows	-	✓	Liver copper levels where not measured, but the group receiving the complex had significantly higher milk production.
Hansen, S.L., *et al.,* 2008	Glycine Complex[7] vs Sulphate	12 Steers	2 - 6	✓	The group receiving copper glycine complex had significantly greater liver copper levels than the group receiving copper sulphate.

[1] Copper lysine complex, Zinpro Corp, Eden Prairie, Mn, USA

[2] Copper proteinate, Bioplex, Alltech Inc, Nicholasville, KY, USA

[3] Copper proteinate, DuCoa, Highland, IL, USA

[4] Copper proteinate, Chelated Minerals Corp, UT, USA

[5] Copper proteinate, Key Minerals Corp, SLC, UT, USA

[6] Copper amino acid chelate, Albion Advanced Nutrition, Clearfield, UT, USA

[7] Copper glycine complex, Pancosma, S.A. (Le Grand-Saconnex, Geneva, Switzerland)

[8] Copper polysaccharide complex, Quali Tech Inc, Chaska, MN, USA

[α] Iron (Ferrous Sulphate)

The copper glycinate injection is copper bound to the amino acid glycine. It has been found that 90% of the elemental copper from copper glycinate reaches the liver (Bohman et al., 1984). Cunningham 1957 administered 400mg elemental copper as copper glycinate to copper deficient hoggets raising liver copper concentrations to within the normal range in 2-4 weeks (Bohman et al., 1984). Toxicity is less of an issue because with this form of copper, although it is released into the blood quickly, it is transferred to blood proteins not free copper ions. Copper glycinate injections can cause a skin reaction in livestock which many farmers do not like thus making them less popular than copper edetate injections.

The copper calcium edetate is the most popular injectable form of copper as it is the safest to use and can provide the greatest level of elemental copper to the animal, and is the longest acting of the three (Wakelin, 1992). Within four hours of injecting with copper the plasma copper concentration peaks and falls again; to around 15.7 μmol/L irrespective of dose rate or whether it is copper EDTA or copper glycinate (Bohman et al., 1984).

A dose of 2ml will supply 100mg of elemental copper and where severe deficiency occurs an 4ml dose can be given to cattle safely (Wakelin, 1992).

Toxicity can still occur however, usually as a result of failure to ascertain the copper status of the herd prior to dosing.

Copper amended fertiliser

Cunningham & Perrin (1946) showed that topdressing with copper sulphate in the autumn reduced the incidence of copper deficiency signs in livestock. Copper sulphate is the most common form of copper used for topdressing, and is often added to the base fertilizer (nitrogen, phosphorus, and potassium etc) boosting the amount of copper which is in the soil increasing the amount which is absorbed by the pasture up into the leaf. The soil is generally seen as deficient in copper at a level of <5ppm (O'Connor, 1992). At a soil copper status of <5ppm an application of anything from 2 kg/ha/year to 8 kg/ha/year

of $CuSO_4$ is required to bring the copper status of the soil to a level which is within a normal range. This is with a capital (initial) application of around 10 kg/ha of $CuSO_4$ (O'Connor, 1992). *Grace* et al., (2004) found that an application of 12kg $CuSO_4$/ha was effective in increasing the copper status of the pasture (figure 6) and of the weanling hinds grazing that pasture. However 6kg $CuSO_4$ /ha did not significantly increase the copper status of the hinds whereas the 12kg $CuSO_4$ /ha did significantly increase the copper status of the hinds (figure 7).

The amount of copper you need to apply as fertilizer is heavily dependent on the soil type, the copper status of the soil, the copper status of the pasture, and the concentration of antagonistic minerals in the soil.

The soil type, or more importantly the exchange capacity of the soil, influences the amount of copper the soil will hold and release (make available) to the pasture. For example a clay soil has a higher exchange capacity than a pumice soil therefore more copper will be held in the clay soil but fertilizers, such as lime, are often needed to release the stored copper. The pumice soil does not have the storage capacity of the clay soil because of its lower exchange capacity but the copper which is applied to the soil will be readily available to the plant. However, leaching of that copper must be taken into account in application rates and the time of the year the copper is applied.

The copper status of the pasture and the concentration of antagonists such as molybdenum should be determined before copper application is performed. If there is high levels of molybdenum in the pasture then a higher level of copper would need to be applied in order that the animal get enough copper to fulfill its requirements. Therefore the concentration of antagonists in the pasture and soil also need to be measured.

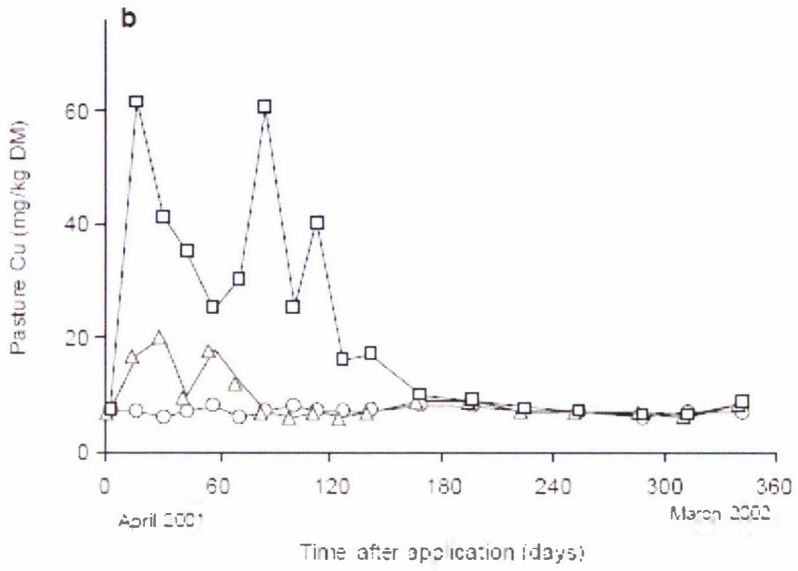


Figure 6. Pasture copper (copper) concentrations per kg dry matter (DM) after no treatment(○), topdressing with 6 kg (△) or 12 kg (□) $CuSO_4$/ha in mid-March 2001–2002 season (Grace et al., 2004).

There are two limiting factors in this method of increasing the copper status of grazing livestock.

The copper status of the soil does not correlate well with the copper status of the pasture because of the many antagonistic factors which exist in the soil reducing the availability of copper to the plant for uptake. Molybdenum is the most common copper antagonist in the soil, and is especially prevalent in wet soils. Iron has a similar action. Because of this antagonist tendency of the soil, topdressing with $CuSO_4$ is not always effective at combating copper deficiency in livestock and is not as cost effective as orally supplementing the animal.

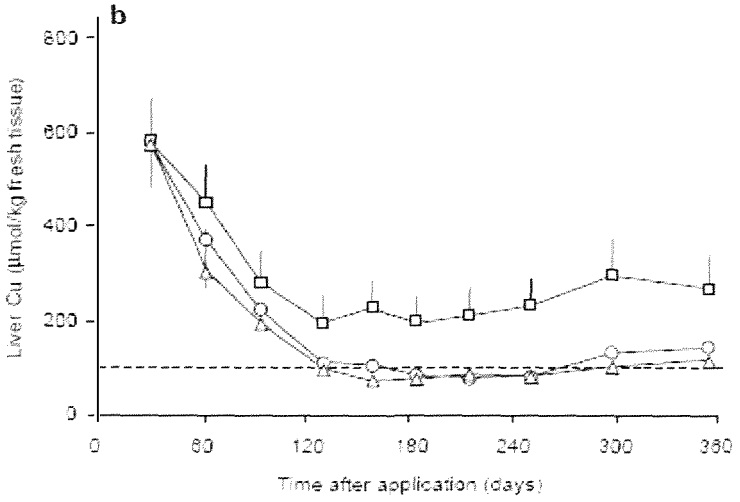


Figure 7. Mean (a) serum, and (b) liver copper (Cu) concentrations of weaner hinds (n=11) grazing untreated pasture (○) and pastures top-dressed with 6 kg (△) or 12 kg (□) copper sulphate/ha in mid-March in Year 1. Standard errors are represented by vertical bars (Grace et al., 2004).

The cost of topdressing with $CuSO_4$ is a further reason why it may not be an attractive option of increasing the copper status of grazing livestock.

CHAPTER 2

MATERIALS & METHODS

MATERIALS & METHODS

Animals

During March and April 2007 approximately 80 Friesian and Friesian, Jersey cross cows where sourced from various dairy farms from the Manawatu, Taranaki areas of the north island of New Zealand. The cows used where of mixed ages, ranging from two and a half to five and a half years old and had an average live-weight of 465kg. They were all cull cows, culled the season of this study, mainly due to infertility. Therefore these cows were a good representation of a New Zealand dairy herd.

During the period of the trial the cows were all grazed together and had access to the same feed, health services, and water. The cows were fed two different forms of feed; pasture and baleage. The diet was made up of approximately 20% pasture and 80% baleage. However, the feed ratios did vary slightly throughout the duration of the trial depending on pasture availability. The herd was split into two main groups of thirty cows, the drenched group and non-drenched group, and divided in the paddock by a fence. The drenched herd was mustered into the shed at the large animal teaching unit every Monday, Wednesday, and Friday at 7:00am, over the duration of the study, and individually drenched based on their identified treatment groups (three treatment groups in the drenching group, and three treatments in the non-drenched group).

Experimental Design

This trial was carried out at the veterinary large animal teaching unit at Massey University, Palmerston North, beginning on 16th July, 2007, and lasting for 4 months. In this study the effect of dosing five different forms of copper on liver and blood levels of copper was investigated in New Zealand dairy cows.

Five treatments were used; three oral treatments and two positive control treatments, along with a control, untreated, group.

The oral treatments where;

Group 1: *copper glycine* (21% elemental copper, 1:1 ratio of glycine amino acid to copper, obtained from Agvance Marketing Ltd, Auckland, NZ) administered three times weekly to supply 150mg of elemental copper per cow per day.

Group 2: *copper amino acid chelate* (10% elemental copper, 3:1 ratio of amino acid to copper, obtained from Agvance Marketing Ltd, Auckland , NZ) administered three times weekly to supply 150mg of elemental copper per cow per day.

Group 3: *copper sulphate* (24% elemental copper, obtained from Agvance Marketing Ltd, Auckland, NZ) administered three times weekly to supply 150mg of elemental copper per cow per day.

Group 4: *copper oxide bolus* (20g bolus, Copacaps, Merial NZ Ltd, Auckland, NZ) administered orally once at the commencement of the trial.

Group 5: *copper injection* (50mg/ml elemental copper in calcium copper edetate form, Bomac Laboratories Ltd, Auckland, NZ) administered subcutaneously to the neck of each animal once at the commencement of the trial and again at day 58.

Sixty cows where used in the trial, ten cows per treatment group. Each animal was assigned its group based on its initial liver copper concentration, thus ensuring the mean liver copper concentrations between the groups where the same. Unfortunately one animal, from the copper glycine group, died and subsequently had to be removed from the trial.

Solution preparation & administration

For the three oral treatments the amount of dry powder for each treatment was weighted out to the nearest 0.01g, to supply 150mg of elemental copper per day, for each week of the four months and placed into a zip lock bag (table 13). Each zip lock bag was separated into its group and labeled with a permanent marker. The amount which was weighed out for each treatment was determined by calculating the elemental copper concentration in each treatment based on three sets of lab analysis of solutions prepared for each of the oral copper treatments prior to the commencement of the trial. The amount of product for each treatment was; copper glycine, 85.4g per litre of water, copper amino acid chelate, 167.2g per litre of water, and copper sulphate, 77.7g per litre of water (table 9). On the Monday of each week each dry powder was put into exactly one litre of de-ionized water making up the solutions for that week. These solutions where contained in plastic four litre drench packs and shaken thoroughly before each drenching. The containers and guns where labeled with the same colour bands as the cows had around their necks so no confusion between the groups in terms of colours could occur. Each container was washed and left to dry at the end of each week, the drench guns were also cleaned and filled with water at the end of the week. On the Monday of each week the new solutions where made up and the water was thoroughly cleared out of each gun before the newly made up solutions were put through the drench guns.

At drenching each cow in the drenching group was given a 20ml drench over the tongue with the treatment solution corresponding to her neck band. The person drenching had three 4 litre containers on a trolley, each containing one of the three oral copper solutions. Every effort was made to ensure that each cow received the correct treatment drench and all of the 20ml drench solution. All the drenching was performed by Shaun Balemi.

Table 9. The amounts of each copper treatment given to the cow (according to its treatment group) over the duration of the trial assuming the CuO Bolus would be spent after the 116 day trial period, and the elemental copper percentage of each copper treatment.

	Total copper product administered per animal				
	Product/Trial (g)	**Product/Week** (g)	**Product/Day** (mg)	**Cu/Day** (mg)	**Elemental Cu** (%)
Cu Glycine	84.87	5.12	731.66	149.99	20.5
Cu Amino	166.28	10.03	1433.46	149.94	10.46
Cu Sulphate	77.25	4.66	665.95	149.97	22.52
CuO Bolus	20.00	1.21	172.41	144.83	84.00
Cu Injection	4.00	0.24	34.48	0.02	0.05

The copper capsule (Copacaps, Merial NZ Ltd, Auckland, NZ) used was medium sized containing 20g of copper oxide needles. One copper capsule was administered to each of the ten cows in the copper capsule group on the 16th July.

The copper injection (copper-max, Bomac Laboratories Ltd, Auckland, NZ) used was in the calcium copper edetate form, commonly used in the New Zealand agricultural industry. A 2ml dose was administered (providing 100mg of elemental copper), under the skin of the neck, to each cow in the copper injection group on the 16th July (day 0), and again on the 27th September (day 58).

Collection of samples

Liver biopsies and blood samples were collected from each animal six times throughout the period of the trial, on day -5, 14, 28, 58, 86, and 116.

The liver and serum samples were analysed by the New Zealand Veterinary Pathology in Hamilton, an independent accredited laboratory.

Liver Biopsy Samples

The hair over the biopsy site was clipped and prepared for surgical procedure with antiseptic solution. 7-10ml of local anesthetic (nopaine 2%) was administered subcutaneously to the area where the incision was made. A small stab incision was then made, approximately 0.5cm long. The biopsy instrument (trocar and needle) was then inserted through the incision punching the diaphragm after which the needle was removed from the trocar allowing the trocar to be inserted into the animal's liver. Once satisfied that the liver had been cored by the trocar a syringe was fitted onto the end of the trocar and drawn approximately 1ml creating suction pressure on the liver sample contained in the trocar. The trocar containing the liver sample, with the syringe attached was then withdrawn from the animal. The cores of liver were approximately 3mm thick and 1-2cm long. The core of live liver was then pushed out of the trocar using the needle onto a fresh paper towel, to absorb any blood from the sample leaving the liver sample exposed. The liver sample was then placed into a sample vial. Each of the sample vials, containing the corresponding cow's number and colour identification, where bagged and submitted to the Veterinary Pathology laboratory, Palmerston North, NZ, the same day the samples were collected.

Blood serum sample collection

These were collected from the coccygeal vein in the tail of the animal, using a needle and vacutainer. The blood samples were then spun in a centrifuge, separating the blood plasma from the serum. Then a sample of the serum was removed from the sample tube using a pipette and placed in a sample vial with the corresponding cow's number and colour group printed on it. The samples where then bagged and sent to the laboratory for analysis on the same day the samples were collected.

Pasture Samples

Three pasture samples were taken over the period of the trial, one on 22nd August, one on 27th September, and the third on 10th November. These were taken by hand, and consisted of a ratio of grasses which reflected the ratio of grasses present in the pasture at the time of sampling. Care was taken not get any soil in the sample. The pasture sample was collected from representative areas, areas which were not largely different, across the paddocks at a spacing of around ten metres. The sample was collected in a paper bag and sent to the Gribbles laboratory, Hamilton, NZ for analysis. The samples were tested for copper, zinc, iron, and molybdenum.

Forage Samples

Three forage samples were taken at the same time as the pasture samples. Samples were taken by hand from different parts of the baleage stack, and mixed together, to give the most accurate representation of what the cows where receiving. The samples were collected in a paper bag and sent to the Gribbles laboratory, Hamilton, NZ, and tested for copper, zinc, iron, and molybdenum.

Analytical Methods

Atomic Absorption Spectrophotometry (AAS) was used to analyse both the liver and serum samples for copper concentration.

The sample was diluted with de-ionized water then a Nitric, Perchloric acid was added to the sample to remove any organic material. From here the sample was subjected to the AAS machine where it was passed through an air-acetylene flame which measured the concentration of copper in the sample. The accuracy of the liver analyses was ±100 µmol/kg fresh weight, at a level of 2400µmol/kg, and for serum samples the accuracy was ±0.4umol/L, at a level of 12µmol/L.

Statistical Analysis

Eighty cows were liver biopsied, pre-study, and the liver copper concentration for each cow determined. The cows with the highest liver copper concentrations were then removed from the herd of study cows leaving sixty cows. These cows had a mean liver copper concentration of 820μmol/kg FW (SE 42.45). By a blocking procedure six groups of ten cows were randomly selected so that each group had a similar mean and variance.

The data collected from this trial was analysed using SAS v9.1 (Statistical Analysis System, version 9.1; SAS Institute Inc., Cary, NC, USA) with a MIXED procedure for repeated measures. The model considered the fixed effects of day of measure (day -5, 14, 28, 58, 86, and 116), treatment group, interactions between day and treatment group, and the random effect of the animal. Means and standard errors were obtained for each treatment group, day of measure and each combination of group and day. Multiple mean comparisons were performed and significant differences were declared at $P<0.05$. Specific contrasts were performed to test significant differences between group effects. However where samples where missed these results and the animal where excluded from that the analysis of the results from that sampling period, however at the next sampling period the animal was reintroduced. Where cows where removed from the trial, due to death, the animal was removed from the entire trial and historical results from that animal were removed from the analysis. This was done to ensure quality of the data. The only other option was to fill in values based on the trends of the other animals in the group this option was decided against in this situation and the animal was excluded from the trial.

Animals were categorized into two groups of initial liver copper concentration; above the mean (high Cu) and below the mean (low Cu). Analyses of variances were carried out on liver copper concentrations over time to detect significant differences between the two initial copper concentration groups, (high copper and low copper) within each treatment group. The model included the fixed effects of day of measure, treatment group and interaction between treatment group and day and the random effect of animal. The response of each animal to copper supplementation and no supplementation was estimated as the slope of the regression line of copper concentration in the liver over

time. Means and standard errors for each of the combination between treatment groups (Control, Cu Glycine, Cu Amino, Cu Sulphate, CuO Bolus, and Cu Injection), and initial copper concentration groups (high Cu and low Cu) within each treatment group, were obtained. Multiple comparisons between initial copper concentration groups within treatment groups were performed and significant differences were declared at $P<0.05$.

Cow 66 from the Glycine group died half-way through the study. Therefore liver copper data from her was unobtainable over the last three sampling periods. It was decided that the results from the first three samplings should be left in the data set because the MIXED procedure accounts for correlations between measures on the same animal throughout the time and data can still be analysed from all the animals, even if records from various animal are missing at some time points. This decision was based on the work illustrated by Wolfinger and Chang (1995).

CHAPTER 3

RESULTS

RESULTS

Pasture and feed supplement analysis

Samples of pasture and baleage where collected three times during the trial, approximately one month apart and analysed for trace mineral and iron concentration.

The baleage analysis showed that the feed was low in mineral content across most of the minerals tested, zinc, copper, cobalt and selenium. The molybdenum, iron, and zinc concentrations were low and therefore unlikely to interact significantly with the copper absorption of the animal. The copper concentration in the baleage was also low thus reducing the level of copper in the diet as baleage made up approximately 80% of the diet.

The pasture analysis showed a reasonably balanced mineral content across most of the minerals tested for. The copper levels of the pasture where surprisingly high at the 10/11/2007 sampling which may have been due to a high clover content of a spring pasture. The 22/08/2007 pasture copper result was considered unreliable and was therefore removed from the table.

Table 10. Results of the pasture and baleage analysis for a full range of minerals, performed by Gribbles Analytical Laboratory, Hamilton, NZ.

Minerals	**Pasture Analysis** (ppm)			**Baleage Analysis** (ppm)		
	22/08/2007	**27/09/2007**	**10/11/2007**	**22/08/2007**	**27/09/2007**	**10/11/2007**
Molybdenum	1.06	1.25	2.2	0.87	1.69	1.39
Iron	719	967	1329	577	184	958
Manganese	186	127	146	84	118	132
Zinc	40	40	44	20	28	25
Copper	-	10	18.9	3.9	7	6.4
Cobalt	0.36	0.45	1.2	0.38	0.27	0.54
Selenium	0.04	0.04	0.06	0.02	0.03	0.03

An evaluation of the efficacy of copper supplements based on changes in liver copper concentrations

The liver copper concentration of the cows from the un-treated control group declined throughout the period of the trial, from a mean initial copper concentration of 827μmol/kg and falling to 554μmol/kg after 116 days (figure 8).

All treatment groups had significantly ($P<0.05$) greater liver copper concentrations than the control group during at least one sampling period of the trial (figure 8 and figure 9). Within the individual sampling periods (day 58, 86, and 116) the three oral treatment groups; Cu Glycine, Cu Amino and Cu Sulphate, had significantly ($P<0.05$) greater liver copper levels than the control group. The CuO Bolus group had significantly greater mean liver copper concentrations than the control group at day 58; however, this result was not consistent throughout the trial, as at no other sampling period was a significant difference from the control group observed (figure 9). Likewise the mean liver copper concentrations of the Cu Injection group were only significantly ($P<0.05$) greater than the control cows over one of the sampling periods (day 86), which was 28 days after the second Cu injection was given (figure 9).

When all the observations over the six sampling periods where collectively analysed the Cu Glycine chelate group showed a significantly ($P<0.05$) higher mean liver copper concentrations than the copper sulphate group (figure 8). The mean liver copper concentration of the Cu amino group was also numerically higher than the sulphate group, but the result failed to reach statistical significance ($P<0.16$).

The three groups receiving the oral Cu Glycine, Cu Amino and Cu Sulphate treatments had significantly ($P<0.05$) greater mean liver copper concentrations than the CuO Bolus and Cu Injection groups over the period of the trial (table 11). In addition the Cu Glycine and Cu Amino groups had significantly ($P<0.05$) greater mean liver copper concentrations than the CuO Bolus and Cu Injection groups from day 86 until the end of the trial (figure 8).

Table 11. The overall changes in the mean (± standard error) liver copper concentration (µmol/kg fresh weight) of dairy cows not supplemented or supplemented with copper over a period of 116 days where each of cows over each of the sampling periods was analysed to reduce variation.

Treatment	Overall	
Control	693[a]	±41
Cu Glycine	1064[d]	±41
Cu Amino	1025[dc]	±40
Cu Sulphate	910[ch]	±42
CuO Bolus	861[b]	±40
Cu Injection	843[b]	±40

[†] Means within rows with differing subscripts are significantly (P<0.05) different.

Figure 8. Changes in the mean (± standard error) liver copper concentration of dairy cows not supplemented (▲), or supplemented, with Cu glycinate chelate (●), Cu amino acid chelate (●) and Cu sulphate (♦), over a period of 116 days.

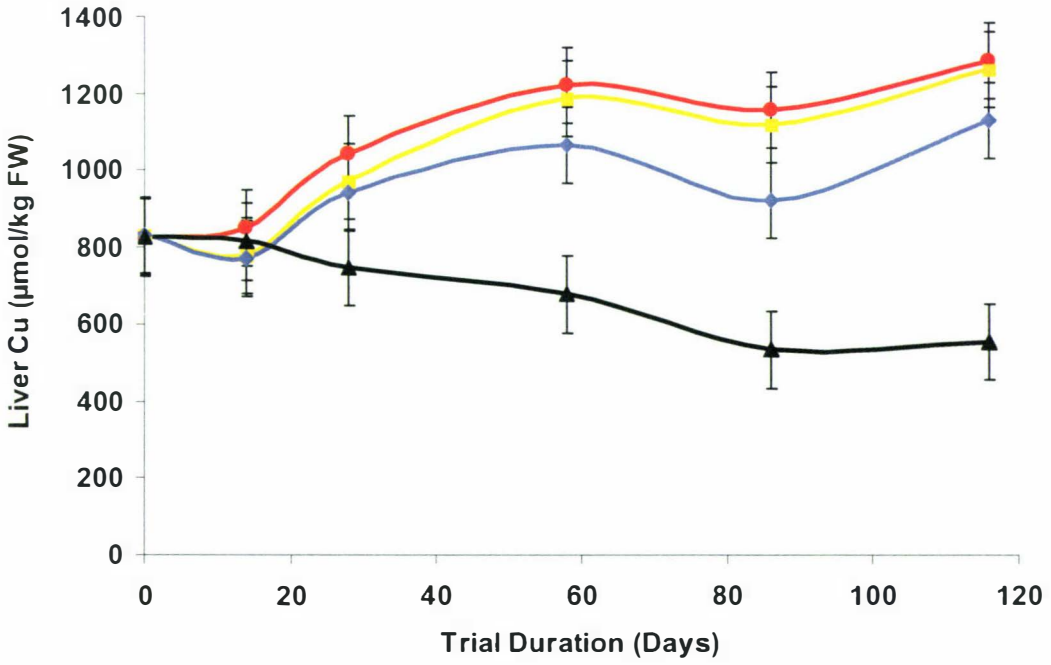
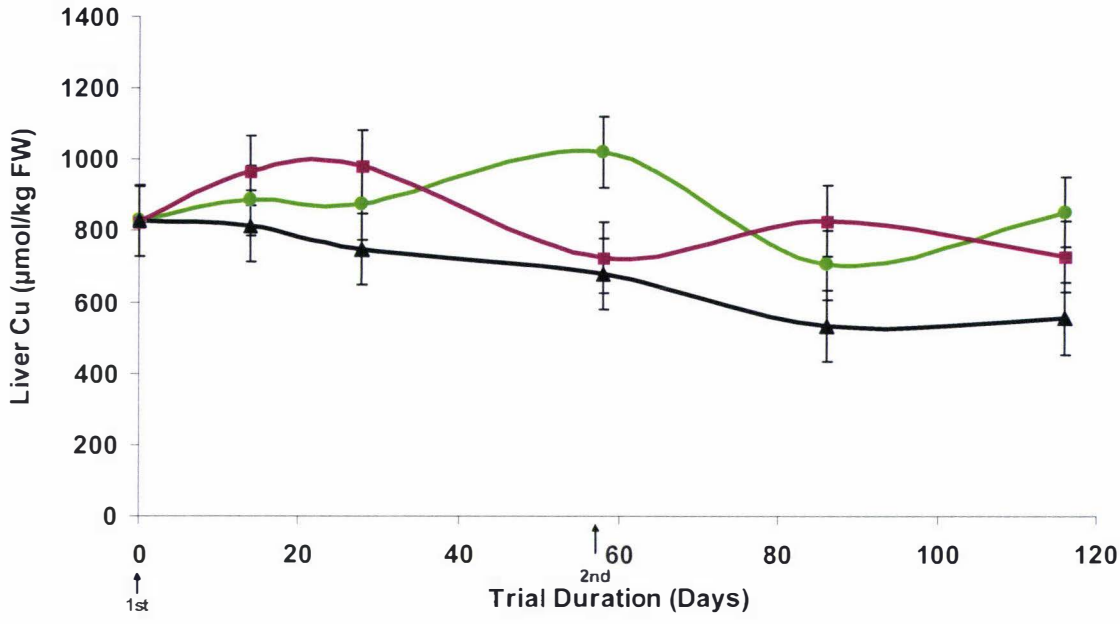


Figure 9. Changes in the mean (± standard error) liver copper concentration of dairy cows not supplemented (▲) or supplemented with, 20g CuO wire particles (●) at Day 1 or two Cu injections (100 mg) administered at Days 0 (1st) and day 58 (2nd) (■), over a period of 116 days.



The mean liver copper concentration of the control group significantly ($P<0.05$) reduced over the 116 day period of the trial, at a rate of -2.35 µmol/kg of liver copper per day (table 12).

In contrast the mean liver copper concentrations of the oral Cu treatment groups, Cu Glycine, Cu Amino and Cu Sulphate, significantly ($P<0.05$) increased over the period of the trial (table 12).

The mean liver Cu concentration of the CuO Bolus group did not increase significantly over the duration of the trial.

The mean liver copper concentration of the Cu Injection group decreased significantly over the duration of the trial, at a rate of -0.22 µmol/kg of liver Cu per day (table 12).

Table 12. The mean (± standard error) initial and final liver copper concentrations (μmol/kg FW) in dairy cows not supplemented, or supplemented, with various copper products over a period of 116 days, and the estimated daily change in liver concentrations.

Treatment	Copper Concentration			
	Initial (μmol/kg)	Final (μmol/kg)	Liver Storage (μmol/kg/day)	P values
Control	827	554[a]	-2.35	<0.001
Cu Glycine	826	1287[c]	3.97	<0.001
Cu Amino	829	1264[bc]	3.75	<0.001
Cu Sulphate	829	1131[bc]	2.6	<0.001
CuO Bolus	827	853[ab]	0.22	NS
Cu Injection	826	728[a]	-0.84	<0.05

Influence of initial copper concentrations on changes in liver copper concentrations

Because there was considerable variation in the initial liver copper concentrations (SE ±42.45) there was the concern that cows with higher (>600μmol/kg FW) liver copper concentrations might respond differently to supplementation compared to cows with lower (<600μmol/kg FW) initial liver copper concentrations. Therefore each cow in the trial, based on initial liver copper concentration, was placed into a higher (>600 μmol/kg FW) initial liver copper sub-group and lower (<600 μmol/kg FW) initial liver copper sub-group. The rate of increase, between the higher and lower initial liver copper concentration groups, over the period of 116 days was then analysed to find if a difference in copper uptake existed between high and low initial liver copper cows. There was no significant difference between cows with higher or lower initial liver copper concentration (table 13, figure 10 and 11).

Similarly there was no significant difference found between the higher and lower initial copper concentration groups within the control group (table 13). However, based on the slope (table 13) the cows within the higher initial copper group appeared to drop to a greater degree than the cows in the lower initial copper group, but the difference was not

significant. Thus the cows with higher or lower initial liver copper concentration responded similarly to supplementation.

Table 13. The mean (±standard error) change in liver copper concentration within the groups, between the cows with high (>600 µmol/kg FW) initial liver copper concentration and low (<600 µmol/kg FW) initial liver copper concentration, when not supplemented or supplemented with 150 mg Cu/day in three different forms over a period of 116 days.

Treatment	Initial Cu Status	Slope	SE	P values
Control	High	-3.1204	±0.94	
	Low	-2.3654	±0.94	NS
Cu Oral	High	2.7306	±0.94	
	Low	4.1961	±0.94	NS

Figure 10. Changes in liver copper concentration in non supplemented dairy cows with either an initially high (>600 µmol/kg FW) (●) or low (<600 µmol/kg FW) (▲) liver copper concentration of 116 days.

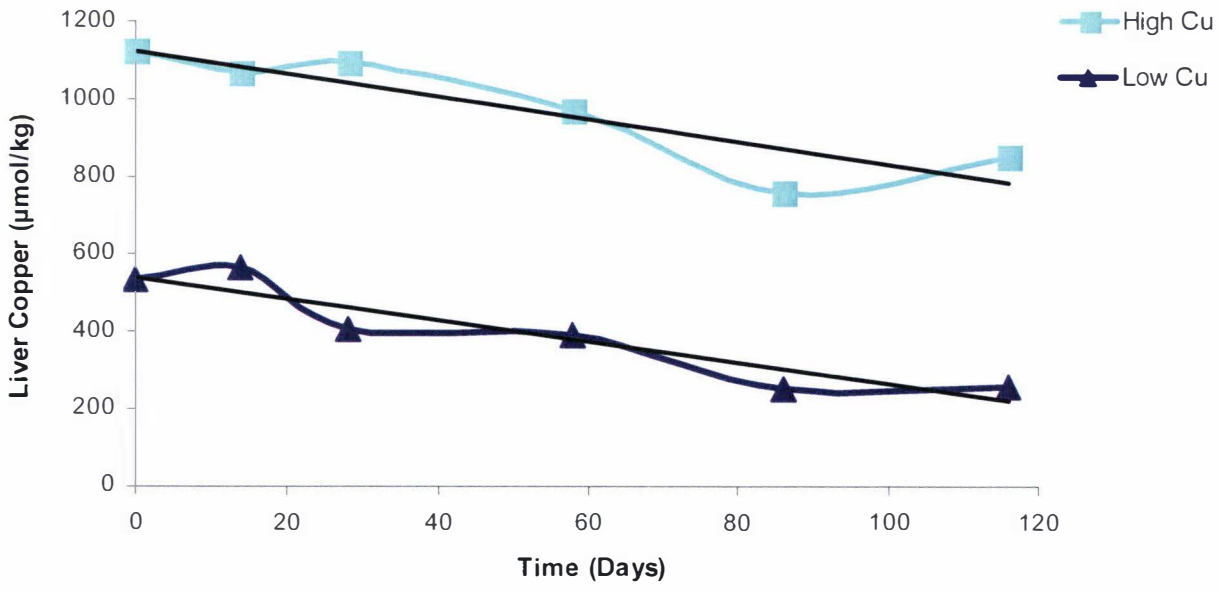
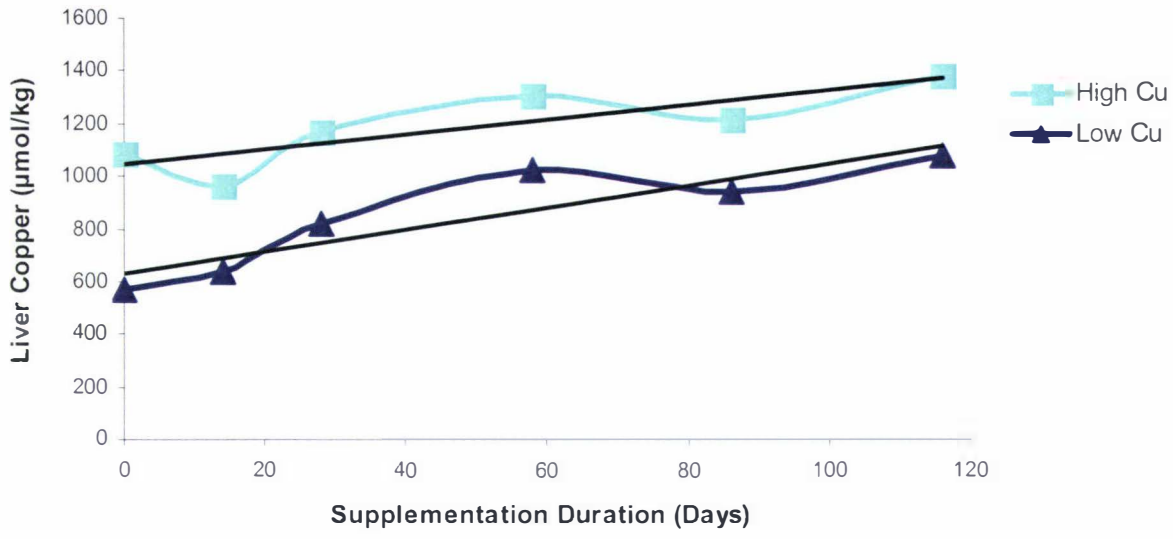


Figure 11. Changes in mean liver copper concentrations of dairy cows with either an initially high (>600 µmol/kg FW) (■) or low (▲) (<600 µmol/kg FW) liver copper concentration and then supplemented with 150 mg of copper as Cu glycinate, Cu amino or Cu sulphate over a period of 116 days.

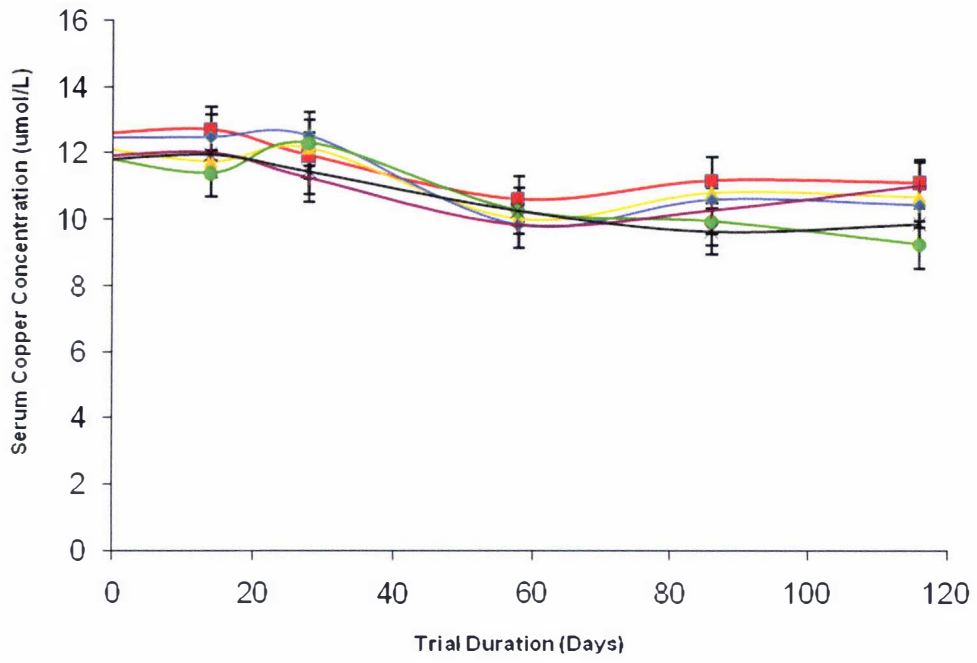


Efficacy of Cu products based on serum Cu concentration

There were no significant differences in serum copper concentrations between supplemented and/or un-supplemented groups over the six sampling periods, except on day 116, when the Cu Glycine group showed a significantly ($P<0.05$) higher mean serum copper concentration than the control group (table 12).

The overall data from the tissue copper determinations showed that the liver copper concentrations reflected changes in animal copper intakes and status while serum copper concentrations did not.

Figure 12. Changes in the mean (± standard error) serum copper concentration of dairy cows not supplemented (x), or supplemented, with Cu glycinate chelate (■), Cu amino acid chelate (△) and Cu sulphate (♦), CuO bolus (●), and a Cu injection (+) over a period of 116 days.



CHAPTER 4

DISCUSSION

DISCUSSION

The importance of copper in New Zealand dairy cows

Copper deficiency in New Zealand cattle was first identified in the early1940's by Ira Cunningham where scouring cattle, farmed on peat soils, responded to copper supplementation.

The current study was conducted in an effort to provide greater knowledge in the copper supplementation of New Zealand dairy cows. This is an area where very little research has been done especially in the area of chelated copper supplements.

Validity of research

Within a herd of cattle, liver copper concentrations can vary, especially where the mean liver copper concentration of the herd is high (West et al., 1997). This proved to be the case in this study where the initial mean liver copper concentration was 827 µmol/kg FW and the initial standard error between cows was 108 µmol/kg FW and none of the animals were copper deficient (<95µmol/kg FW).

Therefore it was important that this variation between animals was taken into account when setting up the trial. This was done by ensuring each group started with a similar mean liver copper concentration ensuring the effect of the variation between each individual animal was minimised. In similar studies this is not commonly done and often animals are placed in groups based on other criteria such as body weight rather than liver copper concentration.

The mean initial liver copper concentration of 827µmol/kg FW was well above the concentration considered to be deficient or requiring supplementation. Therefore a concern was raised as to whether cows with higher (>600µmol/kg FW) liver copper would respond differently to the treatments than cows with lower (<600µmol/kg FW)

initial liver copper concentrations. The responses to copper supplementation of the five cows with the highest liver copper concentrations were compared with the responses to copper supplementation of the five cows with the lowest liver copper concentrations. This was done for each of the treatment groups. It was found that initial liver copper concentration had no effect on the amount of copper absorbed from the copper treatments. Therefore the results gained in this trial are considered to be valid for other dairy herds, even though initial liver copper concentrations may differ from those reported here.

Evaluation of the efficacy of the copper supplements based on changes in liver copper concentration

Each of the oral copper supplements (Cu glycine chelate, Cu amino acid chelate, and Cu sulphate) increased liver copper concentrations, resulting in liver copper concentrations significantly ($P<0.05$) greater than control cows after 58 days of supplementation, with liver copper concentrations continuing to rise over the period of the trial. A dose of 150mg of elemental copper per day was used for each of the three oral treatments. This could be considered a high dose as 150mg is the approximate daily copper requirement for cattle from the diet (Grace, 1994). However, mineral supplements used in New Zealand generally supply between 150 and 250mg of elemental copper per day.

The claims behind chelated copper supplements are that they have a greater bioavailability than non-chelated (or inorganic) copper sulphate. There are studies which support these claims and have found chelated copper to have a greater bioavailability than copper sulphate (Hansen et al., 2008; Nockels et al., 1993). However there are also those that have found that chelated copper did not differ significantly from copper sulphate in bioavailability (Arthington et al., 2003; Rabiansky et al., 1999; Yost et al., 2002). The present study provides some support for the claim that copper chelate has a greater bioavailability than copper sulphate, especially the copper glycine chelate which showed a significantly ($P<0.05$) greater efficacy than the copper sulphate over the duration of the study. In this study the copper glycine was 27 ±14% more efficient in its rate of

absorption and storage in the liver of the cow than the copper sulphate. Hansen (2006) found that copper glycine was 40% more efficient in its rate of absorption and storage in the liver of the cow than copper sulphate.

Many types of copper chelates are marketed as cattle supplements and therefore research on chelates can differ. The copper chelate used by Hansen (2008) was also a copper glycine chelate. However the chelates used by Arthington *et al.*, (2006) and Yost *et al.*, (2002), and Rabiansky *et al.*, (1999) used a copper lysine chelate. Some of these studies found that the copper chelate had a higher bioavailability than copper sulphate and some did not. Therefore variation is found between different copper chelate forms in terms of their bioavailability.

Chelated forms of copper supplements have been found to have greater bioavailability than non-chelated copper sources in the presence of antagonists (Bailey et al., 2001) as the copper ions are believed to be protected from degradation in the rumen and therefore interaction with antagonists such as molybdenum does not occur. This prevents the elemental copper from being made available in the rumen restricting the formation of thiomolybdate compounds which render the copper unavailable to the animal (Underwood & Suttle, 1999).

In this study the concentrations of antagonists, such as molybdenum, were low and further work needs to be done to quantify the effect of antagonists on copper absorption from different forms of oral copper supplements in New Zealand dairy cows.

The cows in the CuO bolus group each received a 20g bolus containing 16.8g of elemental copper. The CuO bolus maintained the mean liver copper concentrations in the cows over the period of the study (116 days) whereas the mean liver copper concentration of the non-supplemented animals dropped significantly. The mean liver copper concentration of the CuO bolus group was 827µmol/kg FW on day 0 and 853µmol/kg FW on day 116. Yost *et al.*, (2002) found that in cows supplemented with a 25g CuO bolus liver copper concentration increased from 20 to 150µmol/kg DM in 28 days, and maintained this liver copper concentration over the 70 day period of the trial. At a liver copper status of 800µmol/kg FW it is of little benefit to the animal to increase liver

copper stores. However based on the marked drop in liver copper concentration of non-supplemented cows it is important for a supplement to maintain liver copper concentration and the 20g CuO bolus achieved this.

The cows in the copper injection group where injected with 2ml of calcium copper edetate, containing 100mg of elemental copper. The liver copper concentration increased within two weeks of administration. Although the mean liver copper concentration increased from 826μmol/kg FW to 981μmol/kg FW the increase did not achieve statistical significance ($P<0.10$). After two months (day 58) the liver copper concentration of these cows had dropped back to the same level as the non-supplemented control animals and therefore it was decided that a second copper injection of the same amount should be given. The second injection caused a significant ($P<0.05$) increase in mean liver copper concentration of the treated group from 726μmol/kg FW to 828μmol/kg FW but decreased to pre-treatment level over a period of approximately 45 days. Similarly Bohman *et al.*, (1984) found that when a calcium copper edetate injection, containing 120mg of elemental copper, was administered to calves, mean liver copper concentration increased from 107 μmol/kg FW to 184 μmol/kg FW within 14 days, showing a rapid uptake of copper into the liver.

Evaluation of the efficacy of the copper supplements based on changes in serum copper concentration

There were no significant differences between the mean serum copper concentrations of the groups receiving the different copper supplements. In this study serum copper concentration did not indicate the copper status of the cattle which is not unexpected as serum copper concentrations have been shown to only reflect liver copper concentration when the liver is depleted of copper, and all the animals used in this study had sufficient liver copper stores (Claypool et al., 1975).

This study showed no significant differences in serum copper concentration over the 116 day period of the trial despite all the groups showing significant differences in liver copper concentration. The reason that no differences were found in the serum collected in

this study as opposed to the liver was that the liver copper stores in all the cows where adequate, therefore the serum copper levels where simply reflecting the amount of copper the animal had collected from the liver and was circulating around the body at that point in time (Mills, 1987).

In practice the liver is a more useful indicator in the determination of the copper status of cattle, which is why it was used in this study. However where blood serum samples are used if the levels are consistently low (<4μmol/L) (Claypool et al., 1975; Suttle, 1976) then this indicates that the liver may be depleted of copper and the herd should be supplemented with copper to avoid signs of copper deficiency.

Decline in liver copper concentration

The consistent decline in the mean liver copper concentration of the control group of cows was not unexpected as others have observed a similar seasonal decline in liver copper in sheep (Grace *et al.*, 2004) and in cattle (West *et al.*, 1997). This study and many other New Zealand studies show that a seasonal decline in liver copper status occurs in cattle from the months of April through to November (winter period). This highlights the need for care when interpreting liver copper analysis when deciding whether to supplement the herd with copper. If liver copper levels are within the normal range in April they may be in the deficient range in 6 months time when the cows are being mated. Therefore accurate prediction of the likely future copper status based on the expected fall in copper status is important, where action must be taken in April to maintain copper levels during this period of copper decline.

Possible impact of increasing molybdenum intakes on the efficacy of copper supplements

High molybdenum levels are considered responsible for inducing much of the copper deficiency seen in New Zealand and may influence the efficacy of copper supplements also. In the resent study molybdenum levels were relatively low (generally <1.5ppm) and

probably had little effect on the amount of copper stored in the liver. It has been suggested that chelated forms of copper may be more efficiently absorbed in the presence of high molybdenum than copper sulphate.

Further work is needed to evaluate the efficacy of chelated forms of copper in the presence of high levels of molybdenum and sulphur in the diet of New Zealand dairy cows.

REFERENCES

Adogwa, A., Mutani, A., Ramnana, A., & Ezeokoli, C. (2005). The effect of gastrointestinal parasitism on blood copper and hemoglobin levels in sheep. *Candian Veterinary Journal, 46*, 1017-1021.

Al-Gubory, K. H., Bolifraud, P., Germain, G., Nicole, A., & Ceballos-Bicot, I. (2004). Antioxidant enzymatic defence systems in sheep corpus luteum throughout pregnancy. *Reproduction, 128*, 767-774.

ARC (Ed.). (1980). *The nutrient requirements of ruminant livestock.* Farnham Royal: Commonwealth Agricultural Bureaux.

Arthington, J. D., Pate, F. M., & Spears, J. W. (2003). Effect of copper source and level on performance and copper status of cattle consuming molasses-based supplements. *Journal of Animal Science, 81*, 1357 - 1362.

Ashmead, H. D. (1993). *The Roles of Amino Acid Chelates in Animal Nutrition*: Noyes Publications.

Ashmead, H. D., & Ashmead, S. D. (2004). The effects of dietary molybdenum sulphur and iron on absorption of three organic copper sources. *The International Journal of Applied Research in Veterinary Medicine, 2*(1), 1 - 9.

Ashmead, H. D., Graff, D. J., & Ashmead, H. H. (Eds.). (1985). *Intestinal absorption of metal ions and chelates.* Illinois: Charles C Thomas Publisher.

Ashmead, H. D., & Samford, R. A. (2004). Effects of metal amino acid chelates or inorganic minerals on three successive lactations in dairy cows. *International Journal of Applied Research in Veterinary Medicine, 2*(3), 181 - 188.

Audige, L., Wilson, P. R., Morris, R. S., & Davidson, G. W. (1995). Osteochondrosis, skeletal abnormalities and enzootic ataxia associated with copper deficiency in a farmed red deer (*Cervus elaphus*) herd. *New Zealand Veterinary Journal, 43*, 70-76.

Bailey, J. D., Ansotegui, R. P., Paterson, J. A., Swenson, C. K., & Johnson, A. B. (2001). Effects of supplementing combinations of inorganic and

complexed copper on performance and liver mineral status of beef heifers consuming antagonists. *Journal of Animal Science, 79*, 2926 - 2934.

Bohman, V. R., Drake, E. L., & Behrens, W. C. (1984). Injectable copper and tissue composition of cattle. *Journal of Dairy Science, 67*, 1468 - 1473.

Bremner, I. (1987). Involvement of metallothionein in the hepatic metabolism of copper. *The Journal of Nutrition, 117*, 19-29.

Bremner, I., & Mills, C. F. (1981). Absorption, transport and tissue storage of essential elements. *Phil. Trans. R. Soc. Lond., 294*, 75-89.

Bruere, A. N. (1982). The clinical diagnosis of copper deficieny/sufficiency. *Proceedings of the New Zealand society of sheep and beef cattle veterinarians of the New Zealand veteriany association, 11*, 15-23.

Claypool, D. W., Adams, F. W., Pendell, H. W., Hartmann, N. A., & Bone, J. F. (1975). Relationship between the level of copper iin the blood plasma and liver of cattle. *Journal of Animal Science, 41*, 911 - 914.

Cunningham, I. J. (1954). Molybdenum and Animal Health in New Zealand. *The New Zealand Veterinary Journal, 2*, 29 - 37.

Cunningham, I. J. (1957). Dual deficiency of copper and cobalt in Hawke's Bay *New Zealand Veterinary Journal, 5*(3), 103-108.

Dewes, H. F., Lowe, M. D., & McKay, C. E. (1990). An assessment of the copper status of dairy herds in the Waikato, Taranaki and Northland in spring and the effects of daily supplementation with copper sulphate. *New Zealand Veterinary Journal, 38*(3), 98-101.

Dowling, A. (1997). Copper deficiency in the wairoa region. *Proceedings of the New Zealand society of sheep and beef cattle veterinarians of the New Zealand veteriany association, 27*(1), 51-57.

Ellison, R. S. (1992). A review of copper and selenium reference ranges in cattle and sheep. *Sheep and Beef, 22*, 3-26a.

Ellison, R. S. (2002). Major trace elements limiting livestock performance in New Zealand. *New Zealand Veterinary Journal, 50*(3), 35-40.

Ellison, R. S., Young, B. J., & Read, D. H. (1986). Bovine post-parturient haemoglobinuria: two distinct entities in New Zealand *New Zealand Veterinary Journal, 34*, 7-10.

Gooneratne, S. R., Buckley, W. T., & Christensen, D. A. (1989a). Review of copper deficiency and metabolism in ruminants. *Canadian Journal of Animal Science, 69*(4), 819-845.

Gooneratne, S. R., Laarveld, B., Chaplin, R. K., & Christensen, D. A. (1989b). Profiles of Cu in blood, bile, urine and faeces from Cu-primed lambs: effect of Mo-labelled tetrathiomolybdate on the metabolism of recently stored tissue Cu. *British Journal of Nutrition, 61*, 355-371.

Grace, N. D. (1975). Studies on the flow of zinc, cobalt, copper and manganese along the digestive tract of sheep given fresh perennial ryegrass, or white or red clover. *British Journal of Nutrition, 34*, 73-82.

Grace, N. D. (1994). *Managing trace element deficiencies*: AgResearch.

Grace, N. D. (2004). *Dietary trace element requirements of dairy cows*. Paper presented at the Society of Dairy Cattle Veterinarians of NZVA.

Grace, N. D., Rounce, J. R., Knowles, S. O., & Lee, J. (1997). Changing dietary S intakes and the Cu status of grazing lambs. *New Zealand Journal of Agricultural Research, 40*, 329-334.

Grace, N. D., Rounce, J. R., & Lee, J. (1996). Effect of soil ingestion on the storage of Se, vitamin B12, Cu, Cd, Fe, Mn, and Zn in the liver of sheep fed lucerne pellets. *New Zealand Journal of Agricultural Research, 39*, 325-331.

Grace, N. D., Wilson, P. R., & Quinn, A. K. (2004). The effect of copper-amended fertiliser and copper oxide wire particles on the copper status of farmed red deer (Cervus elaphus) and their progeny. *New Zealand Veterinary Journal, 53*(1), 31 - 38.

Hafez, E. S. E., & Hafez, B. (2000). *Reproduction in farm animals*: Blackwell Publishing.

Hansen, S. L., Schlegel, P., Legleiter, L. R., Lloyd, K. E., & Spears, J. W. (2008). Bioavailability of copper from copper glycinate in steers fed high dietary sulphur and molybdenum. *Journal of Animal Science, 86*, 173 - 179.

Harris, E. D. (2000). Cellular copper transport and metabolism. *Annual Review of Nutrition, 20*, 291-310.

Hartle, J. W., & Ashmead, H. D. (2006). Bonds important for amino acids chelates. *Feedstuffs, 78*(37), 1-3.

Howell, J. C., & Gawthorne, J. M. (Eds.). (1987a). *Copper in Animals and Man*: CRC Press Inc.

Howell, J. C., & Gawthorne, J. M. (Eds.). (1987b). *Copper in animals and man* (Vol. 1): CRC Press, Inc.

Howell, J. C., & Gawthorne, J. M. (Eds.). (1987c). *Copper in Animals and Man* (Vol. 1): CRC Press, Inc.

Hunter, A. P. (1977). Some nutritional factors affecting the fertility of dairy cattle. *New Zealand Veterinary Journal, 25*, 305-307.

Kincaid, R. L. (1999). Assessment of mineral status of ruminants: A review. *Proceedings of the American Society of Animal Science*.

Klotz, L., Kronche, K., Buckczyk, D. P., & Sies, H. (2003). Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. *journal of Nutrition, 133*, 1448-1451.

Kratzer, F. H., & Vohra, R. (Eds.). (1986). *Chelates in Nutrition*. Florida: CRC Press, Inc.

Laven, R. A., Lawrence, K. E., & Livesey, C. T. (2007). The assessment of blood copper status in cattle: A comparison of measurements of caeruloplasmin and elemental copper in serum and plasma. *New Zealand Veterinary Journal, 55*(4), 171 - 176.

Lee, J., & Grace, N. D. (1997). A New Zealand Perspective on Copper, Molybdenum and Sulphur Interactions in Ruminants. *Proceedings of the society of the sheep and beef cattle veterinarians of the NZ veterinary asscoiation, 27*(1), 25-38.

Lee, J., Masters, D. G., White, C. L., Grace, N. D., & Judson, G. J. (1999). Current issues in trace element nutrition of grazing livestock in Australia and New Zealand. *Australian Journal of Agricultural Research, 50*, 1341-1364.

Legleiter, L. R., & Spears, J. W. (2008). Plasma diamine oxidase: A biomarker of copper deficiency in the bovine. *Journal of Animal Science, 85*, 2198 - 2204.

Lonergan, P., Gutierrez-Adan, A., Rizos, D., Pintado, A., De La Fuente, J., & Boland, M. P. (2003). Relativ messenger RNA abundance in bovine oocytes collected in vitro or in vivo before and 20 hours after the preovulatory luteinizing hormone surge. *Molecular Reproduction and Development, 66*, 297-305.

MacPherson, A., & Gray, D. (1985). Swayback and copper supplementation. *Veterinary Record, 117*, 290 - 291.

Mason, J., Lamand, M., Tressol, J. C., & Mulryan, G. (1988). Studies of the changes in systemic copper metabolism and excretion produced by the intravenous administration of trithiomolybdate in sheep. *British Journal of Nutrition, 59*, 289-300.

McDowell, L. R. (2003). *Minerals in animal and human nutrition* (2nd ed.): Elsevier Health Sciences.

Mills, C. F. (1987). Biochemical and physiological indicators of mineral status in animals: copper, cobalt, and zinc. *Journal of Animal Science, 65*, 1702 - 1711.

Mills, C. F., Dalgarno, A. C., & Wenham, G. (1976). Biochemical and pathological changes in tissues of Friesian cattle during the experimental induction of copper deficiency. *British Journal of Nutrition, 35*, 309-331.

Minatel, L., & Carfagnini, J. C. (2000). Copper deficiency and immune response in ruminants. *Nutrition Research, 20*(10), 1519-1529.

Minatel, L., & Carfagnini, J. C. (2002). Evaluation of the diagnostic value of plasma copper levels in cattle. *Preventive Veterinary Medicine, 53*, 1 - 5.

Neathery, M. W., Rachmat, S., Miller, W. J., Gentry, R. P., & Blackrnon, D. M. (1972). *Effect of chemical form of orally administered Zn-65 on absorption and metabolism in cattle.* Paper presented at the Proceedings of the Society for Experimental Biology and Medicine.

Nockels, C. F., DeBonis, J., & Torrent, J. (1993). Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. *Journal of Animal Science, 71*, 2539 - 2545.

O'Connor, M. B. (1992). Copper and Cobalt Topdressing. *Proceedings of the 22nd Seminar Sheep and Beed Cattle Society New Zealand Veterinary Association*, 121 - 124.

Phillippo, M. (Ed.). (1983). *Trace elements in animal production and veterinary practice* (Vol. 7): The British Society of Animal Production.

Phillippo, M., Humphries, W. R., & Atkinson, T. (1987). The effect of dietary molybdenum and iron on copper status, puberty, fertility and osetrous cycles in cattle. *Journal of Agricultural Science, Cambridge, 109*, 321-336.

Rabiansky, P. A., McDowell, L. R., Velasquez-pereira, J., Wilkinson, N. S., Percival, S. S., Martin, F. G., et al. (1999). Evaluating copper lysine and copper sulfate sources for heifers. *Journal of Dairy Science, 82*, 2642 - 2650.

Sarkar, B. (2000). Copper transport and its defect in Wilson disease: characterization of the copper-binding domain of Wilson disease ATPase. *Journal of Inorganic Chemistry, 79*, 187-191.

Sharma, M. C., Joshi, C., Pathak, N. N., & Kaur, H. (2005). Copper status and enzyme, hormone, vitamin and immune function in heifers. *Research in Veterinary Science, 79*, 113-123.

Singh, S., Prabhakar, S., & Ghuman, S. P. S. (1998). Biochemical status and progesterone during lameness and infertility in dairy cows. *Indian Journal of Animal Sciences, 68*(6), 557-568.

Smith, B. (1972). Preliminary investigations into the copper status of dairy cattle in Northland. *New Zealand Veterinary Journal, 20*(1-2), 56.

Smith, B., & Coup, M. R. (1973). Hypocuprosis: A clinical investigation of dairy herds in Northland. *New Zealand Veterinary Journal, 21*, 252 - 258.

Spears, J. W. (1996). Organic trace minerals in ruminant nutrition. *Animal Feed Science Technology, 58*, 151 - 163.

Spears, J. W. (2003). Trace mineral bioavailability in ruminants. *The Journal of Nutrition, 133*, 1506-1509.

Suttle, N. F. (1974). Recent studies of the copper-molybdenum antagonism. *Proc. Nutr. Soc., 33*, 299-305.

Suttle, N. F. (1976). Predicting the effects of dietary molybdenum and sulphur on the availability of copper to ruminants. *Proceedings of the Nutrition Society, 35*(1), 22A - 23A.

Suttle, N. F. (1991). The Interactions Between Copper, Molybdenum, and Sulphur in Ruminant Nutrition. *Annual Reviews, 11*, 121-140.

Suttle, N. F., Abrahams, P., & Thornton, I. (1984). The role of a soil X dietary sulphur interaction in the impairment of copper absorption by ingested soil in sheep. *Journal of Agricultural Science, Cambridge, 103*, 81-86.

Suttle, N. F., & Angus, K. W. (1978). Effects of experimental copper deficiency on the skeleton of the calf. *Journal of Comparative Pathology, 88*, 137 - 147.

Suttle, N. F., Angus, K. W., Nisbet, D. I., & Field, A. C. (1972). Osteoporosis in copper-depleted lambs. *Journal of Comparative Pathology, 82*, 93 - 97.

Suttle, N. F., & Jones, D. G. (1987). Heinz body anemia in lambs with deficiencies of copper or selenium. *British Journal of Nutrition, 58*, 539-548.

Suttle, N. F., & Jones, D. G. (1989). Recent developments in trace element metabolism and function: Trace elements, disease resistance and immune response in ruminants. *Journal of Nutrition, 119*, 1055-1061.

Tao, T. Y., & Gitlin, J. D. (2003). Hepatic copper metabolism: Insights from genetic disease. *Hepatology, 37*(6), 1241-1247.

Turner, J. C., Shanks, V., Osborn, P. J., & Gower, S. M. (1987). Copper absorption in sheep. *Comparative Biochemistry and Physiology, 86*(No: 1), 147-150.

Underwood, E. J., & Suttle, N. F. (1999). *The Mineral Nutrition of Livestock* (3rd ed.): CABI Publishing.

Underwood, E. J., & Suttle, N. F. (2000). *The Mineral Nutrition of Livestock* (3rd ed.): CABI Publishing.

Vulpe, C. D., & Packman, S. (1995). Cellular copper transport. *Annual Review of Nutrition, 15*, 293-322.

Wakelin, R. L. (1992). Copper supplementation for ruminants. *Proceedings of the 22nd Seminar Sheep and Beed Cattle Society New Zealand Veterinary Association, 145*, 43 - 52.

Wapnir, R. A. (1998). Copper absorption and bioavailability. *The American Journal of Clinical Nutrition, 67*(suppl), 1054-1060.

West, D. M., Vermunt, J. J., & Sargison, N. D. (1997). *Copper supplementation of beef bulls - Benefits can be measured*. Paper presented at the Proceedings of the Society of Sheep and Beef Cattle Veterinarians of the New Zealand Veterinary Association

Wikse, S. E., Herd, D., Field, R., & Holland, P. (1992). Diagnosis of copper deficiency in cattle. *JAVMA, 200*(11), 1625-1629.

Williams, D. M., Scott, W. F., Green, K., & Green, B. G. (1983). Hepatic iron accumulation in copper-deficient rats. *British Journal of Nutrition, 50*, 653-660.

Wilson, P. R., & Grace, N. D. (2001). A review of tissue reference values used to assess the trace element status of farmed red deer (*Cervus elaphus*). *New Zealand Veterinary Journal, 49*, 126-132.

Wolfinger, R. D., & Chang, M. (1995). *Comparing the SAS GLM and Mixed procedures for repeated measures*. Paper presented at the Proceedings of the Twentieth Annual SAS Users Groups Conference.

Xin, Z., . , Waterman, R., Hemken, R. W., & Harmon, R. J. (1993). Copper status and requirement during the dry period and early lactation in multiparous holstein cows. *Journal of Dairy Science, 76*, 2711 - 2716.

Xin, Z., Waterman, D. F., Hemken, R. W., & Harmon, R. J. (1991). Effects of copper status on neutrophil function, superoxide dismutase, and copper distribution in steers. *Journal of Dairy Science, 74*, 3078-3085.

Yost, G. P., Arthington, J. D., McDowell, L. R., Martin, F. G., Wilkinson, N. S., & Swenson, C. K. (2002). Efect of copper source and level on the rate and extent of copper repletion in holstein heifers. *Journal of Dairy Science, 85*, 3297 - 3303.

APPENDIX

Table 14. Changes in the mean (± standard error) liver Cu concentration (μmol/kg fresh weight) of dairy cows supplemented or supplemented with Cu over a period of 116 days.

Treatment	Trial Duration (Days)											
	-5		14		28		58		86		116	
Control	827[a†]	±109	814[a]	±98	748[a]	±99	678[a]	±92	534[a]	±91	554[a]	±114
Cu Glycine[1]	822[a]	±109	854[a]	±98	1064[b]	±99	1221[b]	±97	1157[c]	±91	1287[c]	±108
Cu Amino[2]	829[a]	±109	779[a]	±98	971[ab]	±104	1188[b]	±92	1118[c]	±91	1264[c]	±102
Cu Sulphate[3]	829[a]	±109	770[a]	±98	942[ab]	±99	1066[b]	±92	923[bc]	±86	1131[bc]	±102
CuO Bolus[4]	827[a]	±109	885[a]	±98	873[ab]	±99	1020[b]	±92	706[ab]	±91	853[ab]	±102
Cu Injection[5]	826[a]	±109	968[a]	±98	981[ab]	±104	726[a]	±92	828[b]	±91	728[a]	±102

† Means within rows with differing subscripts are significantly ($P<0.05$) different.

Table 15. Changes in the mean (±standard error) serum copper concentrations (μmol/L) of dairy cows not supplemented, or supplemented, with various copper products over a period of 116 days.

Treatment	Trial Duration (Days)											
(umol/L)	-5		14		28		58		86		116	
Control	11.8[a†]	±0.7	12.0[a]	±0.8	11.5[a]	±0.8	10.3[a]	±0.6	9.7[a]	±0.6	9.9[a]	±0.6
Cu Glycine	12.5[a]	±0.7	12.7[a]	±0.8	11.9[a]	±0.8	10.6[a]	±0.6	11.2[a]	±0.6	11.1[b]	±0.6
Cu Amino	12.3[a]	±0.7	11.8[a]	±0.8	12.2[a]	±0.8	10.0[a]	±0.6	10.8[a]	±0.6	10.7[ab]	±0.6
Cu Sulphate	12.5[a]	±0.7	12.5[a]	±0.8	12.5[a]	±0.8	9.9[a]	±0.6	10.6[a]	±0.6	10.5[ab]	±0.6
CuO Bolus	12.0[a]	±0.7	11.4[a]	±0.8	12.3[a]	±0.8	10.3[a]	±0.6	9.9[a]	±0.6	9.3[ab]	±0.6
Cu Injection	11.9[a]	±0.7	12.0[a]	±0.8	11.3[a]	±0.8	9.8[a]	±0.6	10.3[a]	±0.6	11.0[ab]	±0.6

† Means within rows with differing subscripts are significantly ($P<0.05$) different.

Table 16. Raw liver copper data on each of the cows over the 6 sampling events.

Cow No.	Group	11072007 Liver	30072007 Liver	13082007 Liver	12092007 Liver	10102007 Liver	09112007 Liver
24	Control	270	228	206	146	95	135
2	Sulphate	275	231	418	552	540	694
23	Glycine	330	580	902	1098	940	928
22		350	353	421	652	550	698
43	Bolus	363	920	624	1060	740	705
40	Injection	364	380		343		589
56	Control	372	209	287	226	170	150
8	Sulphate	378	352	585	785	530	934
13	Glycine	387	487	576	702	730	929
62		419	455	723	947	1100	1032
50	Injection	435	795	817	823	790	788
65	Bolus	437	345	391	395	400	362
31	Bolus	441	377	417	630	570	525
26	Injection	478	702	684	490	660	567
55	Bolus	566	584	588	960	600	947
76		588	696	1039	1214	1000	955
32	Sulphate	603	675	725	959	800	841
80	Glycine	609	782	1117	1251	1200	1458
38	Control	611	929	312	585		
4	Control	663	724	567	455	380	326
17	Injection	677	945	832	699	700	302
7		725	661	704	1290	810	1204
34	Glycine	738	997	1154	1283	1200	1204
71	Sulphate	738	871	989	1123	1100	1082
52	Control	745	720	647	535	370	417
42		755	871	961	1175	1200	1309
29	Bolus	756	1123	1235	1306	740	1191
37	Injection	791	1067	1071	740	940	699
54	Glycine	807	799	1053	1305	1400	1864
21	Sulphate	831	701	895	1015	960	968
18	Sulphate	840	847	1318	1335	1500	1531
12		874	1053	1375	1243	1500	1957
3	Injection	903	1005	852	869	770	893
27	Bolus	946	799	895	858	850	752
44	Control	970	796	1114	833	790	928
11	Sulphate	973	868	845	1274	1100	1501
15	Control	974	963	966	805	660	613
46	Glycine	985	909	881	1172	1300	1157
41	Injection	1007	1278	1180	846	940	760

6	Sulphate	1011	754	1106	1111	1100	1295
60	Bolus	1011	1102	1286	1530		1210
28	Control	1035	805	953	825	710	
33	Injection	1041	1044	1038	1033	880	897
69		1044	977	1254	1306	1400	1450
25	Glycine	1063	912	1238	1388	1200	1286
47		1070	704		1047	1100	1062
51	Glycine	1119	1015	1117	1135	840	864
39	Bolus	1135	1036	1083	1020	770	748
30		1144	1054	1105	1573		1162
35	Control	1224	1135	1143	1139	760	1007
14	Sulphate	1231	1154	1116	1022	860	1276
9	Injection	1245	1366	1373	1038	1400	1226
74	Bolus	1282	1210	1046	1350	680	1073
1	Injection	1318	1097	981	375	370	562
48		1325	965	1156	1431	1400	1810
19	Bolus	1332	1350	1167	1095	1000	1016
64	Glycine	1359	1202	1541	1657	1600	1890
79	Control	1409	1631	1283	1234	870	859
68	Sulphate	1410	1250	1424	1481	740	1191

Table 17. Raw serum copper data on each of the cows over the 6 sampling events.

Cow No.	Group	11072007	30072007	13082007	12092007	10102007	9112007
24	Control	13	16.1	13.8	12.6	12	12.7
2	Sulphate	11	13.2	10.5	10.6	11.1	12
23	Glycine	12	12.6	11.4	10	12.2	11.8
22		13	12.8	14	9.3	11.5	9.8
43	Bolus	15	15.2	15.1	12.1	10.3	10.7
40	Injection	14	12	11.2	9.1	8.3	9.4
56	Control	15	16.1	16.4	11.9	10.2	11.7
8	Sulphate	9.4	8.5	20	8.7	9.6	10
13	Glycine	13	12.4	11.6	11.7	11.6	12.8
62		12	11.8	14	9.9	8.7	9.6
50	Injection	10	8.8	10.7	10.3	10.1	9.8
65	Bolus	11	11	12.4	10.1	8.7	6.5
31	Bolus	11	12.1	12	10.4	9.3	9.7
26	Injection	13	11.4	12.2	10.8	10.7	12.8
55	Bolus	14	13.1	12.9	10.7	10.5	9.7
76		12	10	9	8.9	8.9	8.8
32	Sulphate	11	10.7	9.3	10.1	10.9	8.4

80	Glycine	13	12.5	11	9.6	8.7	10.4
38	Control	14	13.4	13.4	12.1	10.5	10.5
4	Control	8	8.3	7.5	5.4	8.5	7.6
17	Injection	13	9.1	10.5	7.9	9.9	9.9
7		13	12.3	12.3	9.7	17.5	14.1
34	Glycine	12	10.7	10.6	9.9	11.7	12.1
71	Sulphate	9.6	10.2	8.3	9.1	7.9	8.1
52	Control	6.7	6.7	6.8	7.7	6.1	6.4
42		16	14.9	12.1	11.8	11.3	12
29	Bolus	11	8.7	11.3	7.6	7.4	8.3
37	Injection	13	11.9	11	10.8	11	14.7
54	Glycine	17	10.8	9.6	8.6	10.1	9.1
21	Sulphate	17	13.7	14.1	9.4	11.9	10.3
18	Sulphate	16	16.3	15	10.1	10.3	10.1
12		12	14.6	17	11.1	13	15.8
3	Injection	9.7	12.6	10.3	8.1	10.2	9.9
27	Bolus	13	11.1	13.7	10.1		9.8
44	Control	14	12.5	12.2	11.8	10.7	10.1
11	Sulphate	14	15	12.6	10.9	12.1	13.3
15	Control	12	11.7	11.7	10.6	11.3	11.8
46	Glycine	12	11.4	9.5	9.1	8.5	8.5
41	Injection	11	9.8	9.1	8.5	9	8.1
6	Sulphate	8.7	9.4	8.7	6.6	8.9	9.5
60	Bolus	13	15.4	14.4	16.2	13.2	13.4
28	Control	13	11	10.3	7.8	8.4	8.3
33	Injection	11	12.7	12.5	11.1	11.2	10.8
69		11	5.7	12.1	10.2	9.7	10.1
25	Glycine	7.9	16.1	16.2	10	12.5	13.2
47		14	13.2	13.4	11.4	9.5	10.1
51	Glycine		11.1	9.3	9.4	10.3	9.2
39	Bolus	12	9.6	11.8	10.1	12.1	8.9
30		9.7	11	9.4	8.5	10.3	8.7
35	Control	11	13.3	12.5	11.8	9.4	10.8
14	Sulphate	13	14.7	14.2	11.6	10.9	12.1
9	Injection	12	14.6	10.8	9.1	10.7	12.4
74	Bolus	11	8.8	10.1	7.2	7	6.3
1	Injection	12	17.2	14.2	12.7	11.7	12.5
48		9.9	11.2	8.3	9.4	7.7	7.8
19	Bolus	8.9	9	9.4	8.2	11	9.2
64	Glycine	15	16.5	16.9	17.3	15.1	13
79	Control	11	10.6	10	11.1	9.4	8.9
68	Sulphate	15	13.2	12.6	11.4	12.5	10.7