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Predicting copper status of cattle remains an enigma

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ABSTRACT

Data are presented from several studies carried out by AgResearch and Massey University describing scenarios that affect the Cu status of grazing cattle. In all these studies, complex interactions between Cu, Fe, Mo and S make it difficult to predict Cu status. Increasing pasture Mo reduced percent absorption of dietary Cu in sheep by half (from 4.6% down to 2.6%). In contrast, the Cu kinetics in cattle could not be similarly simplified, so more sophisticated modelling calculations need to be applied. Even with moderate Mo intake (i.e. from pasture 0.8 mg Mo/kg DM), liver Cu content was quickly depleted in the cattle, reaching a low but stable level of 8 mg Cu/kg DM that appears to be a Mo-insensitive liver Cu store. In other trials, various Cu supplementation strategies have produced considerable but unexpected responses in liver Cu content. The risks and benefits of using liver Cu concentrations as an index of Cu status, and whether a multi-variable approach using tissue biomarkers, animal physiology, diet composition, and time of exposure is what is required to accurately predict the future Cu status for cattle are discussed.

Keywords: Copper metabolism; stable isotopes; copper supplements; molybdenum; ruminants.

INTRODUCTION

Detection of the early stages of micronutrient deficiency is one of the most challenging themes of micronutrient science. In the case of copper (Cu), gross deficiency is clinically recognisable and hence treated through supplementation (Grace, 1983; Underwood & Suttle, 1999), but identifying the more common marginal deficiency is often problematic. Interactions between Cu with other micronutrients, confounded by variations in protein and energy supply or through parasite challenge, can mask the extent of the problem (Underwood & Suttle, 1999), and make quantifying an economically significant Cu deficiency difficult.

The current criteria used to assess the Cu status of cattle are liver and serum Cu concentrations of <45 µmol/kg fresh weight (FW) and <4.5 µmol/L respectively, indicating deficiency, and >95 µmol/kg FW and >8 µmol/L indicative of adequate Cu. While, growth responses to Cu supplementation have been reported below 95 µmol/kg FW (Grace, 1994), normally growth responses are not expected until liver Cu concentration falls below 45 µmol/kg FW.

Absorption and tissue bioavailability of dietary Cu is typically very low, partly from poor solubility in the anaerobic environment of the rumen, which is further depressed by interactions with molybdenum (Mo). The formation in the rumen of poorly absorbed thiomolybdate [Cu-Mo-(S)sulphur] complexes results in an induced Cu deficiency and low Cu status, which in turn is linked with bone and nervous disorders, poor liveweight gain and impaired reproductive performance (Mason, 1981; Gooneratne *et al.*, 1989).

We have previously described the effect of increasing pasture Mo concentration, in the presence of dietary S, on absorption and storage of Cu by grazing sheep and showed that Mo concentrations as low as 1 mg Mo/kg DM in pasture have a significant impact on Cu absorption (Knowles *et al.*, 2000). Thus in order to diagnosis Cu insufficiency, the animal's Cu status plus any factors that might induce a deficiency must be identified.

Despite much research locally and internationally, the dynamics of Cu absorption, storage and utilisation by cattle at pasture in New Zealand remain unclear and outcomes are often unpredictable. Compared to sheep, cattle are more sensitive to low Cu intake and the effects of induced Cu deficiency. Current thinking is that Cu concentrations in liver must be high enough in autumn to counter a winter depletion and still meet the high Cu demands of growth, pregnancy or lactation. Beyond that general recommendation, however, the wealth of accumulated evidence on Cu metabolism and dietary interactions in grazing animals has yet to yield a more definitive prescription of how to identify and manage cattle Cu status.

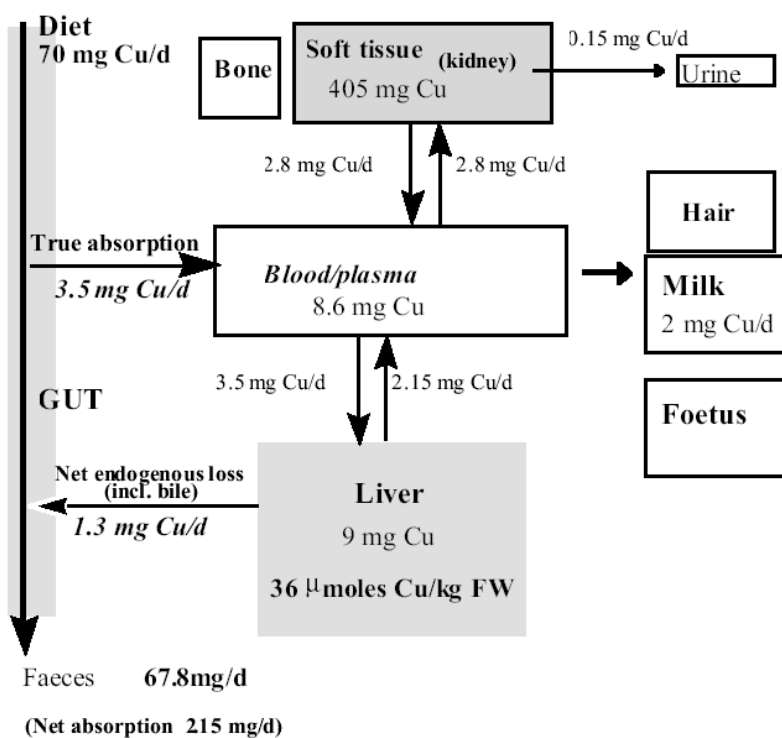
COPPER METABOLISM

In order to help predict the impact that nutritional factors and interactions have on Cu absorption and consequently on an animal's Cu status, it is useful to understand the key elements of Cu metabolism. For a typical hill country lactating Angus cow (liveweight 450 kg and producing 10 L of milk per day), a generalised factorial model for the metabolism of Cu is illustrated in Figure 1. The model is based on data from cattle in the Wairoa district of New Zealand (Litherland *et al.*, 1999) and pools and fluxes of Cu through the body. For simplicity, steady state has been imposed on the tissue compartments as the animal is neither gaining nor losing weight and the transfer of Cu between pools follows first-order kinetics. In reality, both compartment sizes and fluxes will be continuously changing.

In the depicted model, Cu requirements are met, and therefore any decrease in the absorption coefficient and/or dietary intake will require mobilisation of Cu from the liver to meet the Cu demands of other tissues. In this example, liver Cu concentration is only one-third the recognised threshold indicating Cu adequacy (36 vs. 95 µmol Cu/kg FW liver), and as we contend later in the paper, further reduction in liver Cu is more difficult.

Daily output by excretion or secretion includes

FIGURE 1. A generalised factorial model for the metabolism of Cu based on typical hill country lactating Angus cow (450 kg live weight, producing 10 L of milk per day).



endogenous loss (e.g. secretions through saliva, bile, erosion of intestinal cells) and irretrievable loss via milk, urine and hair (the latter is comparatively small and has not been accounted). The extent to which Cu in some of these endogenous secretions are resorbed is not known, although resorption of Cu in bile is thought to be low. Lactation in cows does not raise Cu requirements significantly (Underwood & Suttle, 1999). Urinary Cu output for cattle (Buckley, 1991) is generally less than 2% of the daily flux leaving the liver. Few tracer studies have been conducted to measure endogenous loss in cattle or sheep, so there is uncertainty on the magnitude of this loss from the body.

The absorption and utilization of dietary Cu is sensitive to dietary antagonists, notably iron (Fe), Mo and S. Molybdenum intake in the presence of S markedly reduces Cu absorption, storage and utilisation, through synthesis of thiomolybdates in the reticulorumen and formation of insoluble Cu thiomolybdates in the digesta and tissues (Suttle & Field 1983). Historically, most of our insight into how Mo, S and Cu interact has been based on sheep fed indoors on defined diets. From this earlier work, predictive regression equations derived from pasture concentrations of Cu, Mo and S, and Cu concentrations in liver, show pasture Mo as the main factor linked to Cu deficiency (Grace, 1994; Suttle, 1996; Underwood & Suttle, 1999). Many farmers, consultants and veterinarians are aware of these interactions, but those relationships based on experimental diets are not always applicable to New Zealand grazing animals. Furthermore, Cu absorption is also impaired through interactions with

Fe and Zn, and dietary Fe through soil ingestion (Grace & Lee, 1990). A new approach for New Zealand is called for, because effective risk management strategies will require experimental data and recommendations appropriate for the fresh forage of grazing cattle and sheep.

CASE STUDIES AND EXPERIMENTS

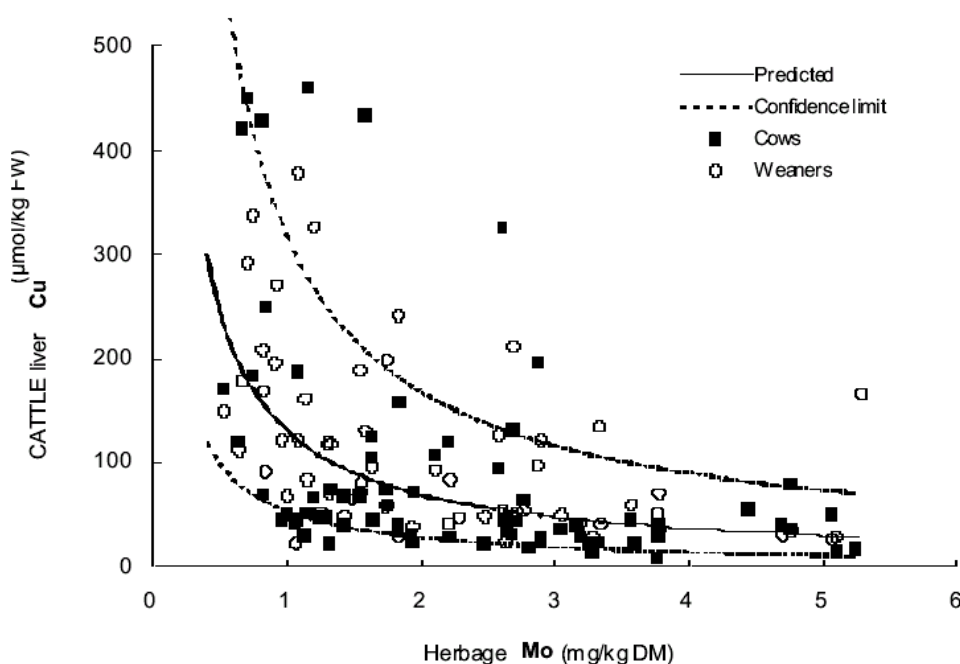
Copper related problems in the Wairoa region

Korte *et al.* (1995) surveyed concentrations of Cu in liver tissue from young weaned cattle and cows on 100 farms on the East Coast of New Zealand and investigated associated factors influencing Cu status. The main findings from that study were:

- Copper concentration in liver varied over an order of magnitude (20-470 μmol Cu/kg FW) with 30% showing deficient Cu status (<45 μmol/kg FW).
- Predictive regression equations relating concentrations of Cu, Mo, and S in the pasture to liver Cu identified pasture Mo as the most significant factor linked to the observed Cu deficiency (see Figure 2).
- Herbage Mo concentrations as low as 0.5-1.0 mg Mo/kg DM in spring were associated with low concentrations of Cu in the liver, a significant proportion of which were below the recognised criteria for Cu adequacy (95 μmol Cu/kg FW).
- This concentration of Mo was much lower than previous guidelines used to assess the impact of Mo in pasture on Cu metabolism.

In a follow-up study of 7 farms in the Wairoa region (Litherland *et al.*, 1999), the seasonal changes in pasture Cu, Fe, Mo and S, pasture growth and soil moisture were

FIGURE 2. Relationship between the concentration of Cu in liver and Mo concentration in pasture for weaner cattle and cows in the Wairoa region (Korte *et al.*, 1995). Solid line is predicted fit for all data and dotted lines are the 90% confidence intervals.



measured and their relationships with liver Cu concentration in weaners and breeding cows were determined. In summary the key points were:

- Concentrations of Cu in liver of younger cattle were generally lower than in the cows, and although many animals had liver Cu below 95 $\mu\text{mol Cu/kg FW}$, clinical signs of Cu deficiency were not observed.
- Variation in pastures Cu and Mo between farms was large, while seasonal variation in Mo was small, and Cu, Fe and S showed significant seasonal variation with the highest concentrations in autumn and winter.
- Although Cu in pasture was usually $>8 \text{ mg Cu/kg DM}$, the Cu/Mo ratio varied markedly between farms.
- Copper fertiliser was applied on one farm (which raised pasture Cu in June/July period to $>15 \text{ mg Cu/kg DM}$), but liver Cu concentrations in both young cattle and cows were low.
- The correlation in liver Cu between livestock classes was frequently poor, so the Cu status of any one class should not be used to infer the Cu status of another.
- Injections of Cu-glycinate had only a short temporal effect on increasing and maintaining liver Cu concentrations.
- Throughout the survey area, pasture Mo is implicated in reducing Cu absorption, but the effect is not a simple one and may be confounded with other dietary and/or metabolic effects.

As concluded by Korte *et al.* (1995), pasture Mo concentrations as low as 0.5 mg/kg DM may impair absorption of Cu, and concentrations of 1-2 mg Mo/kg DM have major impact on animal Cu status, but once Mo rises above 2 mg Mo/kg DM then any further reduction in Cu absorption is minimal.

Copper supplementation studies with bulls

Friesian bulls grazing a lowland farm in the Manawatu were identified as having low liver Cu content, and trials were conducted to investigate effective ways of supplementation (West & Sargison, 1998). Giving 20 g CuO capsules (Cuprax, Schering-Plough Animal Health Ltd, Upper Hutt) elevated liver Cu stores for about 10 months and resulted in a small but significant live weight gain of about 12 kg. Much of the weight gain occurred during late spring when liver and serum Cu concentrations of unsupplemented bulls were in the deficient reference range (i.e. $<45 \mu\text{mol/kg FW}$ and $<4.5 \mu\text{mol/L}$). Liver Cu concentrations fell each year over the winter-spring period but it is not clear as to what caused this decline in Cu status. Pasture Cu concentrations remained relatively constant at 7 to 9 mg Cu/kg DM, Mo concentrations increased from 0.18 mg Mo/kg DM in June to 1.4 mg/kg in October, and Fe concentrations also increased over winter to 1440 mg Fe/kg DM, the latter probably as a result of soil contamination.

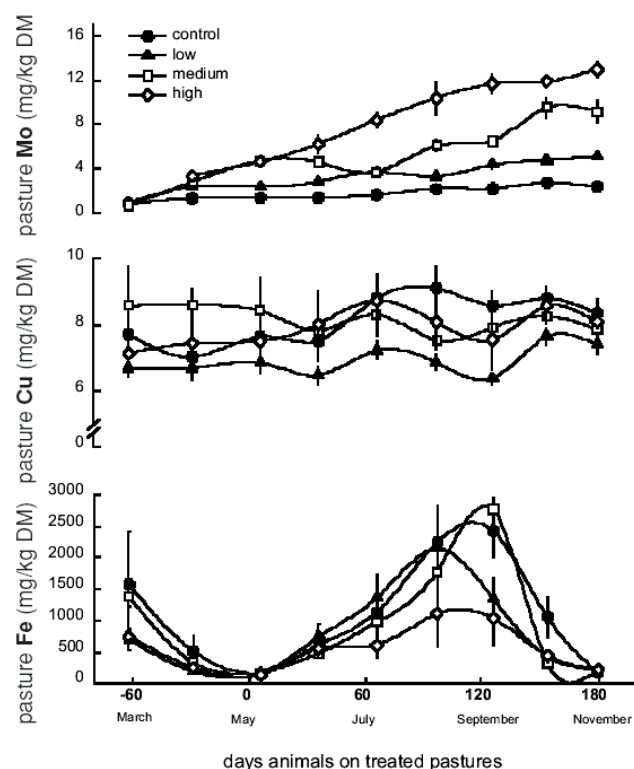
In the following year, 19 ha of the same farm was dressed with 6.3 kg copper sulphate per ha and changes in liver Cu concentrations of bulls grazing this area was assessed by liver biopsy. A separate group of bulls were supplemented with 20 g CuO capsules as before or with 2 ml injection of calcium Cu EDTA salt providing 100 mg Cu (Coprin; Schering-Plough Animal Health Ltd, Upper Hutt). Dressing with Cu increased pasture to 23-25 mg Cu/kg DM for at least 6 weeks, but this was not reflected in liver Cu content of the bulls grazing that pasture. The mean liver Cu concentration of the bulls on the Cu dressed area continued to decline in a similar way to the unsupplemented control bulls, although they eventually

stabilised at a higher level. The Cu topdressing may have been beneficial in the longer term, but it is not clear why the liver Cu concentrations did not increase when the bulls were consuming herbage containing 25 mg Cu/kg DM. Pasture Fe and Mo concentrations were below 500 mg Fe/kg DM and 0.6 mg Mo/kg DM respectively at this time. In contrast there was a significant elevation of liver Cu stores of bulls given a 20 g CuO capsule. Why this form of oral Cu supplementation was effective but grazing pasture recently dressed with Cu was not, remains an enigma.

Measurement of Cu absorption using stable isotope experiments

In a replicated experiment carried out at AgResearch Ballantrae Research Station near Woodville, New Zealand, whether lambs and weaner cattle grazed together for about 180 days on Mo-fertiliser-treated pastures containing 1 to 12 mg Mo/kg DM, in order to quantify the effect of Mo on Cu kinetics of animals in a controlled trial. Changes in animal Cu status were monitored by plasma and liver biopsy Cu and Mo concentrations, plasma caeruloplasmin/ferroxidase activity, and by growth rates. In another facet of this trial dietary Cu absorption was determined using a stable isotope ^{65}Cu tracer technique. Details of experimental protocols are reported elsewhere (Knowles *et al.*, 2000). The Mo treatments were designated as:

FIGURE 3. Seasonal changes and the effect of applying Mo fertiliser to the Mo, Cu, and Fe concentrations of pastures grazed by cattle and sheep. Pastures were treated with control (●), low (◻, 250-500 g Mo/ha), medium (◻, 1000 g Mo/ha), or high (◻, 1500 g Mo/ha) amounts of Mo-amended fertiliser. Points represent treatment mean \pm SEM. Note the different scales.



control (6 paddocks), low (5), medium (2) and high (4).

As shown in Figure 3, pre-treatment herbage Mo concentration was 0.8 ± 0.1 mg Mo/kg DM, and all treated pastures showed continuous steady increases. Pasture Cu was not affected by time or treatments (averaging 7.8 ± 0.1 mg Cu/kg DM), but pasture Fe varied with season, rising as much as 9-fold during winter. Pasture S fluctuated somewhat with herbage turnover, but averaged 3200 mg S/kg DM. These four elements directly impact Cu metabolism, through formation of various CuMoS and FeS compounds in the rumen.

Two months after the Mo fertilisers was applied, groups of weaner cattle matched for similar initial liver Cu stores were distributed on the experimental paddocks. Liver biopsies and blood samples were collected throughout the trial. Changes over time in total liver and plasma Cu and Mo concentration, are shown in Figure 4. On day 68 the cattle received either 5 or 10 mg of ^{65}Cu (depending on initial liver Cu concentrations) via 0.5-hour i.v. infusions. Isotope ratio ^{65}Cu : ^{63}Cu measurements in liver biopsies and plasma were made to determine isotope enrichment, from which tracer kinetics were determined (Figure 5).

From an initial 12 μmol Cu/L, plasma Cu concentration in all cattle decreased throughout the 180 day trial, but the effect of Mo was greatest during the first 60 days in cattle grazing the higher Mo pastures. The pattern of changes in caeruloplasmin activity was similar to that of plasma Cu, and these two measures of Cu status were very significantly correlated ($R^2 = 0.89$; $P < 0.001$). Liver Cu stores were quickly depleted in cattle, reaching a low but stable level of about 35 μmol Cu/kg FW by 80 days. This remaining Cu (about 20% of initial liver Cu) appears to be a Mo-insensitive liver Cu pool, being relatively unaffected even after prolonged Mo exposure.

The concentration of Mo in liver tissue was mostly unaffected by grazing Mo-treated pastures; averaging 6 μmol Mo/kg FW in cattle. Blood plasma Mo, very rarely reported for grazing animals, showed a dose response to treatments (range 0.05 to 2.0 μmol Mo/L). A transient doubling of plasma Mo occurred during September, which corresponded to the seasonal increase in Fe intake. As plasma Mo concentrations appear to be quite sensitive to absorbed Mo, we propose that this value may become an important diagnostic of Mo intake.

Using stable isotope tracer ^{65}Cu in sheep we showed that increasing pasture Mo concentration from 2 to 12 mg Mo/kg DM reduces the amount of Cu absorbed by sheep from 4.6% to 2.2% of dietary Cu intake (Knowles *et al.*, 2000). However, in weaner cattle, we were not able to use the same single compartment (liver) exponential calculation as for sheep to estimate percent absorption, so more sophisticated modelling calculations will need to be applied.

This experiment provided a unique opportunity to compare Cu metabolism between species. The results revealed species similarity as well as some startling differences. Cattle Cu status was more sensitive to Mo and showed smaller Cu reserves, but no live weight loss or clinical signs of Cu deficiency were observed in either species until the very end of the trial (cattle scouring).

Average weight gain was 490 g/d for cattle, with no significant difference among treatments. A longer lasting deficiency however might have reduced growth and fertility, as would any additional stresses which put demands put on Cu nutrition and metabolism, such as rapid growth, inadequate pasture, stock movement, or reproduction and lactation. The scope and scale of our experiment precluded testing for very long term effects, but definitive work at the Rowett Research Institute has shown direct and induced effects of high Mo intake over 1 to 2 years, including delayed puberty, lower weight at puberty, and lower rate of pregnancy (Phillippo *et al.*, 1987). This implies a significant risk to cattle farmers who breed their own replacement stock.

DISCUSSION AND CONCLUSIONS

The Cu x Mo interaction in the presence of sulphur is a more important factor in the Cu nutrition of cattle, and particularly of young cattle, than was previously appreciated. At relatively low, and commonly occurring, pasture herbage concentrations of 0.5 - 2 mg Mo/kg DM, dietary Cu absorption is impaired, the levels of blood indices of Cu status are reduced, and liver Cu stores are markedly depleted. In grazing animals, both time and concentration are important determinants of cumulative

FIGURE 4. Effects of increasing pasture Mo concentration on the concentration of Cu and Mo in liver and plasma. Pastures were treated with control (●), low (△), medium (□), or high (◇) amounts of Mo-amended fertiliser. Points represent treatment mean ± SEM. Horizontal lines mark deficiency criteria. Note the change in scale.

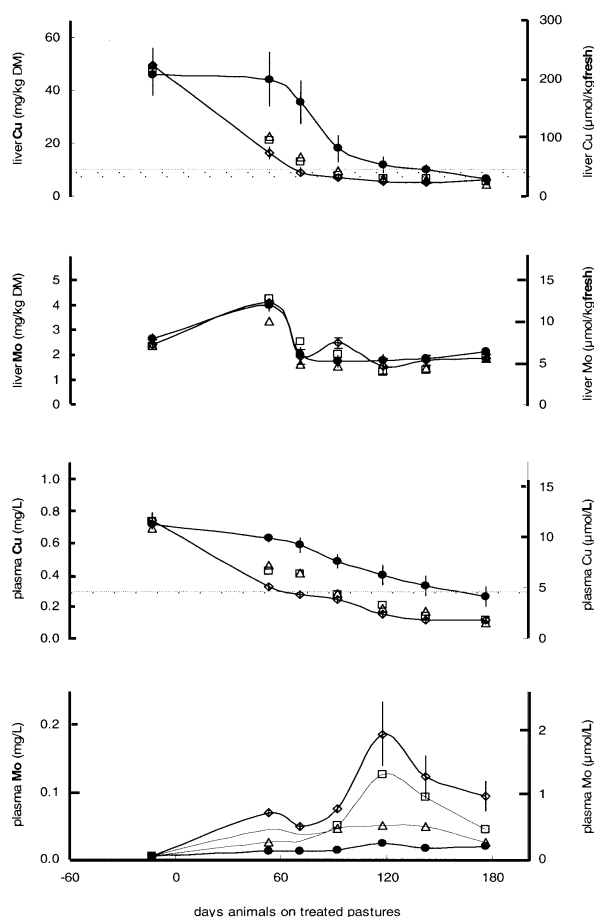
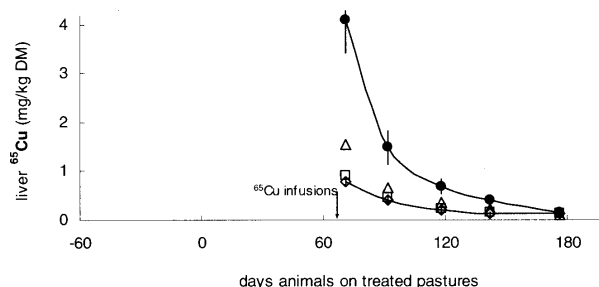


FIGURE 5. Effects of increasing pasture Mo concentration on the rates of disappearance of ⁶⁵Cu tracer from liver of cattle, following infusion of 5 to 10mg ⁶⁵Cu on day 68. Pastures were treated with control (●), low (△), medium (□), or high (◇) amounts of Mo-amended fertiliser. Points represent treatment mean ± SEM.



Mo exposure, and less of one will offset more of the other. Pastures require small amounts of Mo to support clover growth, and 0.3-0.5 mg Mo/kg DM in mixed pasture is typically recommended. Pasture Mo concentration can change over seasons, and more than one herbage sampling and analysis is recommended. And certainly determination of only Cu in pasture is not adequate for assessing animal Cu status. In the case of the bull studies the form of Cu, either in the diet or in oral supplements was influential in changing the Cu content of liver. In this case elevated Cu in the herbage did not increase liver Cu, in contrast to the oral supplements. The poor Cu absorption in fresh forage may be linked to high crude protein, concomitant with high S, as Cu absorption is higher in drier, summer pastures compared with spring pasture. However Grace & Lee (unpublished data) observed that fast growing lambs absorbed up to 10% of Cu in dry lucerne pellets containing relatively high crude protein. Although any concentration of Mo in the presence of S will have an effect on Cu metabolism in grazing livestock, the Cu nutrition of animals is unlikely to be affected by content of <0.5 mg Mo/kg DM, and little measurable impact on growth of young cattle will occur over 8 months at <1-2 mg Mo/kg.

Cattle will experience a “winter” depletion of liver Cu stores when grazing pastures of greater than 1-2 mg Mo/kg DM, with changes in pasture Fe and S being other key factors. Ensuring high liver Cu concentrations in cattle in autumn (i.e. >600 μmol/kg FW, 150 mg Cu/kg DM) to counter the combined effects of winter depletion and a spring season increase in pasture Mo concentration, may still not guarantee an adequate Cu status (>95 μmol/kg FW, 25 mg Cu/kg DM) for mating in the spring. This is because dietary factors driving Cu and Mo absorption have a major effect on clearance of Cu from the liver. In fact, kinetic models based on Figure 1 show that Cu liver stores deplete to a minimal quantity regardless of the starting pool size or Cu absorption coefficients, within approximately the same time frame. The isotope study indicated a Mo-insensitive liver Cu pool that was relatively unaffected even after prolonged Mo exposure. It is not known if this pool is readily available to meet Cu metabolic requirements, but in the longer term is likely to be insufficient. In cases of severe Cu deficiency, treatment with quick-acting injectable Cu may be

necessary, but this strategy is unlikely to maintain Cu status for as long as 'slow release' dietary supplements, because of rapid Cu clearance from the liver driven by stored and circulating thiomolybdates. To date, the best management strategy, as highlighted by the bull beef study, is a proactive supplementation with 10-20 g CuO needles. In some cases dressing pastures with Cu (copper sulphate) in the autumn may be an option but current recommendations should be received.

ACKNOWLEDGEMENTS

This research was supported by Meat New Zealand and Foundation for Science, Research and Technology to provide information that will benefit the New Zealand Meat Industry.

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