Copper fertiliser increases pasture copper concentration and improves the copper status of grazing sheep

S.O. KNOWLES[1], N.D. GRACE[1], J.R. ROUNCE[1], D.M. WEST[2] and J. LEE[1]
[1]AgResearch, Grasslands Research Centre, PB 11008, Palmerston North
[2]Institute of Veterinary, Animal and Biomedical Sciences, Massey Univ, PB 11222, Palmerston North

Abstract

Application of copper-amended fertiliser ("topdressing") increases pasture herbage Cu concentration and can increase dietary Cu intake of grazing ruminants. Animal responses to dietary Cu, in terms of changing blood and liver Cu concentrations monitored throughout the season, have not been well documented. In this study 10 experimental paddocks were topdressed with 0 (Control), 0.4 (Low) or 4.0 (High) kg Cu/ha as $CuSO_4.5H_2O$, resulting in mean herbage Cu concentrations of 8, 13 and 41 mg Cu/kg DM, respectively. The treated pastures were significantly greater than controls for at least 100 days. Romney lambs of low but not deficient initial Cu status grazing the Cu-treated paddocks for 176 days had increased Cu intake compared with controls, resulting in substantial accumulation of Cu in liver. Maximum liver Cu concentration occurred after 99 days, when the levels in low- and high-treated sheep were 3 to 12 times greater than controls (900 to 3270 vs 270 mg Cu/kg fresh tissue). No changes in blood plasma Cu concentration were observed. A predictive relationship between pasture Cu and liver Cu concentrations of grazing lambs was determined, and recommendations for sheep Cu supplementation via Cu topdressing are made.

Keywords: copper status, copper supplementation, liver biopsy, Romney lambs, topdressing

Introduction

Cattle, sheep and deer in New Zealand grazing pastures low in copper (Cu) and high in molybdenum (Mo) risk Cu deficiency. This can be prevented by suitable Cu supplementation strategies, including treatment with orally administered CuO needles, Cu injections, and the application of Cu to pastures with Cu (Grace 1994). Topdressing application of 2.5 to 20 kg $CuSO_4.5H_2O$/ha has been shown to increase pasture Cu content from 4 to 20 mg Cu/kg DM, and can maintain these Cu concentrations for 9 to 12 months (Cunningham & Perrin 1947). Factors such as season, soil type, pasture composition, and rate of Cu application will influence Cu uptake by herbage, and ultimately affect plant Cu concentration and availability of Cu to the grazing animals (Adams & Elphick 1956; Mitchell *et al.* 1957; Sherrell & Rawnsley 1982). The effect of Cu application on pasture and its impact on dietary Cu intake of ruminants has yet to be fully characterised.

This study describes changes in the Cu status of Romney sheep grazing for 6 months on pastures of low to high Cu content, via monitoring concentrations of Cu in liver and plasma.

Materials and methods

As part of a larger study to determine the relationship between Cu intake and Cu status of grazing lambs (Grace *et al.* 1998), pastures were topdressed with various amounts of copper sulphate. Copper as $CuSO_4.5H_2O$ was thoroughly mixed with superphosphate fertiliser and the Cu-amended fertiliser applied by hand in mid April at the rate of 250 kg/ha to 10 experimental paddocks of mean area of 0.8 ha. The treatments were superphosphate with no additional Cu (Control); superphosphate plus 0.4 kg Cu/ha (1.6 kg $CuSO_4.5H_2O$/ha; Low); superphosphate plus 4.0 kg Cu/ha (16 kg $CuSO_4.5H_2O$/ha; High), on 3, 3 and 4 paddocks, respectively.

Samples of herbage were gathered from all paddocks in early April to determine pre-experimental concentrations of Cu and other elements. About 2 weeks later, the fertiliser treatments were applied. This was designated as Day 1 of the study. Further herbage samples were collected on Days 30, 65, 99, 141 and 374.

A flock of 100 8-month-old Romney lambs was used in this study. Fifty of these lambs were sourced from a Raupunga farm in the Wairoa district and had low but not deficient initial Cu status (13 mg (205 μmol) Cu/kg fresh liver tissue). The animals were transported to Ballantrae Research Station near Woodville and for 4 weeks before the experiment were grazed as a single flock. Liveweights were recorded on Days 1, 50, 141 and 175 of the study. On Day 17, the experimental lambs were eartagged, weighed, randomly assigned to experimental paddocks, and set stocked at 12 animals/ha. Two lambs from each paddock, about one fifth of the animals, were designated as monitor animals and liver biopsy samples were obtained from these on Day 1 and again on Days 51, 99 and 142. Blood was collected from 4 lambs per paddock on Days 17, 51, 99, 142 and 175. On Day 176, all lambs were slaughtered at a local abattoir.

Collection and analysis of samples

Herbage samples (400 g fresh pasture) were collected along the same 100–150 m transect in each paddock by cutting the sward down to 10 to 15 mm every 5 to 7 m. Sub-samples were freeze-dried and ground before analysis for Cu and other elements. Blood was collected from the jugular vein into 7 ml heparinised vacutainers specially prepared for trace element analysis. Following centrifugation, the plasma was removed and stored at -20°C. Liver samples of 50–100 mg were obtained by the modified biopsy procedure of Dick (1944) under general anaesthesia. Samples were washed in 0.9% NaCl, patted dry, and stored at -20°C.

Elemental composition of pasture, liver and plasma was determined by inductively coupled plasma (ICP) emission spectrometry (Lee 1983). In preparation for ICP analysis, freeze-dried samples (0.5–1.0 g) were digested in concentrated Analar HNO_3 and the digest residue dissolved in 2*M* HCl. One-ml samples of plasma were digested in 0.5 ml concentrated Analar HNO_3 and 0.5 ml 30% H_2O_2 in polyethylene tubes in a waterbath for 2 hours at 90°C.

Data were analysed by ANOVA and Duncan's multiple-range test, using procedures of Statistical Analysis System (SAS 1987). Liver Cu concentration data were subjected to $\log_{10}$ transformation before statistical analysis because of heterogeneity of variance among treatments.

Results

By Day 30 Cu concentrations of herbage from treated pastures were significantly increased over pre-experimental levels, and remained greater than those of controls for 99 and 141 days for the low-treated and high-treated pastures, respectively (Figure 1). By Day 374 Cu concentrations had returned to baseline values. As no data are available to describe accurately pasture Cu responses during the latter half of the year, a simple gradual decline is estimated. From Days 30 through 141 the mean herbage Cu concentrations were 8, 13 and 41 mg Cu/kg DM for control, low-treated and high-treated pastures, respectively.

Liver Cu concentration in animals grazing control pastures varied slightly with season but was not significantly changed from initial levels at the end of the 6-month trial (Figure 2). By Day 51 liver Cu concentrations in treated lambs were significantly increased over pre-experimental levels, and remained greater than those of control animals through slaughter on Day 176. Increasing mean pasture Cu concentrations resulted in substantial accumulation of Cu in liver. Maximum liver Cu concentration occurring on Day 99, when the levels in low-treated and high-treated sheep were three to 12 times greater than controls. There was no effect of pasture Cu treatment on blood plasma Cu concentrations; mean plasma Cu concentration for all

Figure 1 Effect of application rate of Cu-amended fertiliser and season on the concentrations of Cu in mixed ryegrass/clover pasture over 12 months (mean ± SEM, n ≈ 3). ❍ Control (0 Cu); ☌ Low (0.4 kg Cu/ha); ❒ High (4.0 kg Cu/ha).

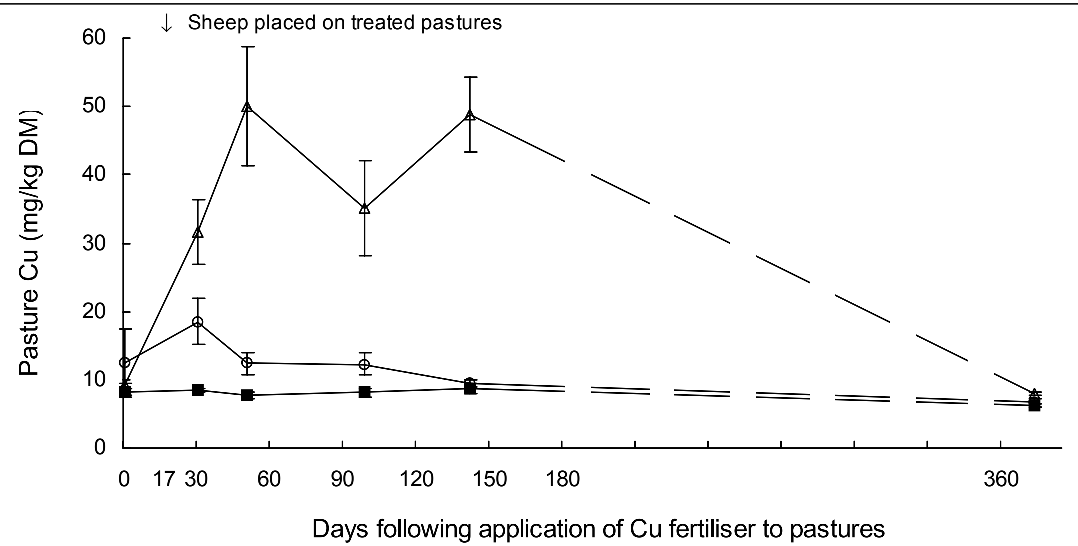


Figure 2 Effect of application rate of Cu-amended fertiliser and season on liver Cu concentrations in grazing Romney sheep over 176 days (mean ± SEM, n ≈ 3 for Days 0–142; n ≈ 15 for Day 176). O Control (0 Cu); Low (0.4 kg Cu/ha); ❒ High (4.0 kg Cu/ha).

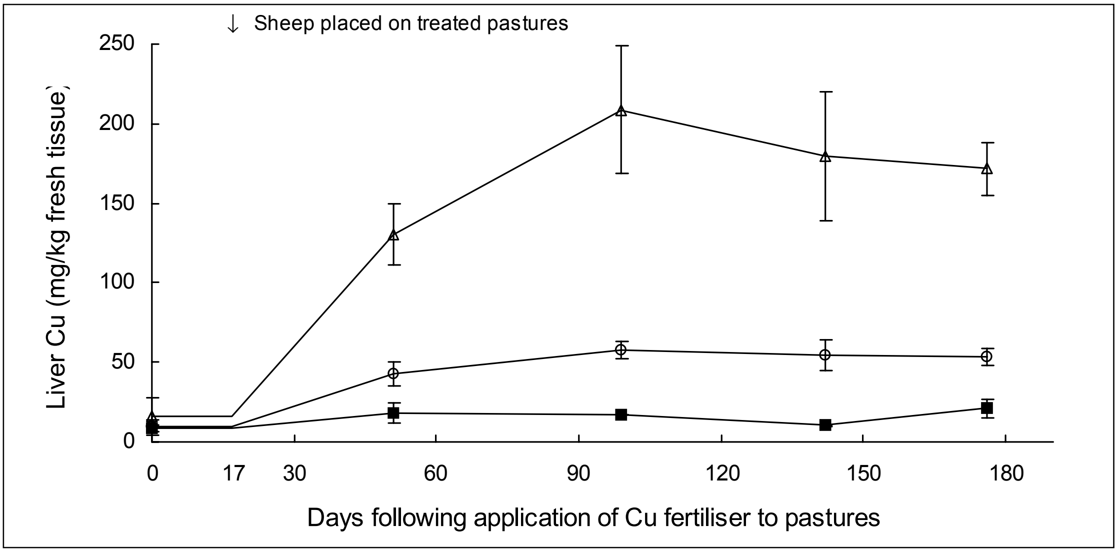
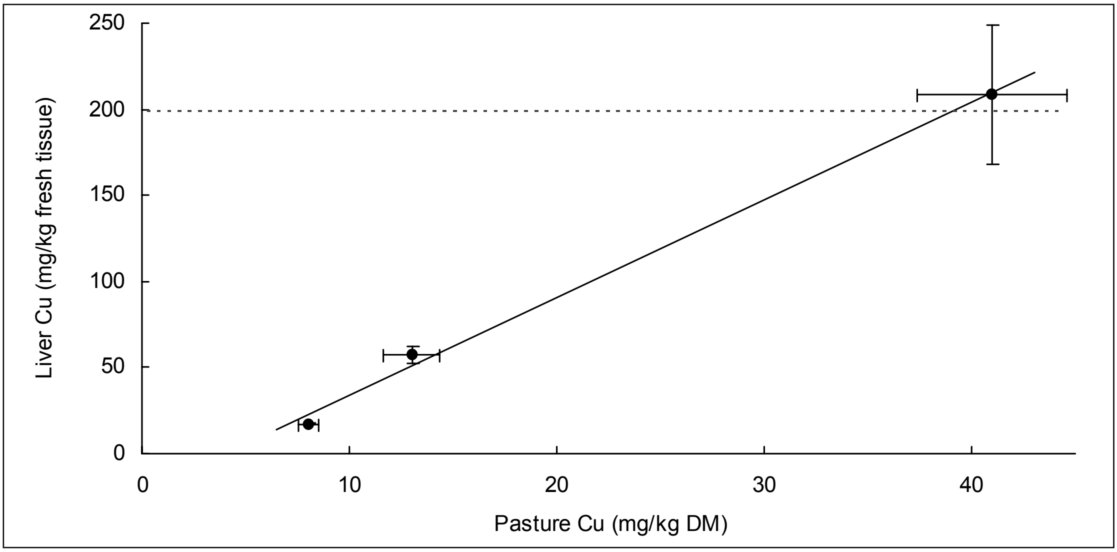


Figure 3 Relationship between mean pasture Cu concentration (Days 30 through 141; x) and maximum liver Cu concentration (Day 99; y), y = 5.3 ± 1.17x – 15 ± 32 (R^2 = .72, n=10, P<0.001). Horizontal line at 200 mg Cu/kg fresh tissue indicates maximum permissible limit of Cu in organs for human consumption (NZ Food Regulations 1984).



sheep during the trial was 0.79 ± 0.036 mg Cu/l (12.3 ± 0.63 µmol/l). Likewise, Cu treatments did not affect animal growth. The mean initial and final liveweights of all animals were 31 kg and 47.4 ± 0.67 kg, respectively, and the average daily weight gain was 93 ± 3.8 g/day.

Pasture Cu (x) was a good predictor of liver Cu concentration (y) in grazing Romney sheep. As shown in Figure 3: y = 5.3 ± 1.17x – 15 ± 32 (R^2 = 0.72, n = 10, P<0.001).

Discussion

Copper application raised Cu content of herbage on treated pastures and markedly improved the Cu status of grazing sheep, as assessed by liver Cu concentrations.

Based on pasture Cu concentrations (Figure 1) and estimated mean daily dry matter intakes of 1.6 kg/day for 39 kg lambs gaining 100 g/day (Sykes & Nicol 1983), mean Cu intakes were calculated to be 13, 21 and 65 mg Cu/day for animals on the control, low-treated and high-treated pastures, respectively. These Cu intakes were reflected in liver Cu concentrations. Sheep with liver Cu concentration less than 6 mg Cu/kg fresh tissue are Cu deficient (Grace 1994). When Cu intake and absorption exceed metabolic demands of the tissues, the excess Cu accumulates in liver. Liver stores are not simply sequestered but are utilised to maintain tissue Cu levels when dietary Cu intake or absorption are inadequate. Thus the effectiveness of a supplementation strategy such as Cu topdressing is extended beyond the period of increased herbage Cu concentration because of the carry-over effect of liver Cu stores. For example, the Cu concentration of pastures treated with 0.4 mg Cu/ha was greater than that of control pastures only through 99 days (Figure 1), but liver Cu concentration of the grazing sheep was increased and maintained above that of controls for at least 176 days (Figure 2). The rate of mobilisation of Cu from the liver depends on Cu dietary intake, absorption, tissue demands, age, breed and any additional metabolic requirements, for example, when animals are pregnant or lactating. Also, interaction of Cu with other elements, particularly Mo and sulphur, can interfere with adequate Cu absorption and utilisation (Lee & Grace 1997). At Ballantrae the pasture Mo concentration was low, averaging 1.1 ± 0.07 mg/kg DM for all the experimental paddocks.

In contrast, too much Cu is toxic. If accumulation in the liver exceeds 450 mg (7000 μmol) Cu/kg fresh tissue, stress factors can trigger a massive release of Cu into the bloodstream, resulting in increased blood Cu concentration, rapid haemolysis, increased blood glutathione and serum enzymes such as aspartate aminotransferase (AST/SGOT), and increased kidney Cu concentrations (Todd 1969).

Dietary Cu requirements of lambs range from 3 to 4 mg Cu/kg DM (Grace 1994), and diets are generally recommended to contain less than 20 mg Cu/kg DM, otherwise chronic Cu toxicity can occur. In this study, Romney lambs grazed pastures of mean Cu concentration 41 mg Cu/kg DM for 176 days without any clinical signs of Cu toxicity being observed, and their average weight gain was 93 g/day, which is considered satisfactory for North Island hill country. Excessive Cu application should be avoided, however, because the maximum permissible limit (MPP) for liver Cu is 200 mg (3130 μmol) Cu/kg fresh tissue (NZ Food Regulations 1984), and liver and kidneys exceeding the MPP must be rejected for human consumption.

Elemental analysis is usually necessary to accurately monitor and adjust Cu application, as herbage Cu concentration resulting from Cu topdressing will be affected by season, pasture composition and soil type. Greater quantities of Cu may not lead to longer-lasting effects. Application of Cu at typical recommended rates of 1 to 2 kg Cu/ha per annum, which should result in pasture Cu concentrations of 10–20 mg/kg DM, would have acceptable margins of efficacy and safety (Figure 3), provided that the applied Cu is washed from herbage into the soil (cf. West *et al.* 1997).

In this experiment Romney lambs served as the model animal, but significant differences in Cu metabolism for other sheep breeds have been reported. In the UK, for instance, Welsh Mountain sheep, when compared with Scottish Blackface, were found to have higher plasma Cu (0.91 v. 0.61 mg Cu/l) and higher liver Cu concentrations (200 vs 100 mg Cu/kg DM) because the Welsh Mountain are more efficient at absorbing Cu (Woolliams *et al.* 1983; Woolliams *et al.* 1985). Also, Texel sheep have been shown to be quite sensitive to Cu poisoning (Woolliams *et al.* 1982). In New Zealand, where the Texel is becoming popular as a terminal sire, grazing Texel lambs on recently Cu-treated pasture should be done with caution.

This study supports a recommendation that topdressing pastures with 1 to 2 kg Cu/ha (4 to 8 kg of $CuSO_4.5H_2O$) as a safe and effective means of increasing the Cu intake of grazing animals.

ACKNOWLEDGEMENTS

Supported by the New Zealand Public Good Science Fund (Contract No. C10622) and Meat New Zealand (Contract No. 96PR–31/1.5).

REFERENCES

Adams, A.F.R.; Elphic, B.L. 1956. The copper content of some soils and pasture species in Canterbury. *New Zealand journal of science and technology 38A*: 821–836.

Cunningham, I.J.; Perrin, D.D. 1947. Copper compounds as fertilisers for pastures deficient in copper. *Department of Agriculture publication 36*. Wellington, New Zealand Department of Agriculture.

Dick, A.T. 1944. Aspiration biopsy of the liver in sheep. *Australian veterinary journal 20*: 298–303.

Grace, N.D. 1994. Managing trace element deficiencies. AgResearch, Palmerston North.

Grace, N.D.; Knowles, S.O.; Rounce, J.R.; West, D.; Lee, J. 1998. Effect of increasing pasture copper concentrations on the copper status of grazing Romney sheep. *New Zealand journal of agricultural research 41*: 377–386.

Lee, J. 1983. Multi-element analysis of animal tissue by inductively coupled plasma emission spectrometry. *ICP information newsletter 8*: 553–561.

Lee, J.; Grace, N.D. 1997. A New Zealand perspective on copper, molybdenum and sulphur interactions in ruminants. pp. 25–38. *In:* Proceedings from the 27th Seminar of the Society of Sheep and Beef Cattle Veterinarians. New Zealand Veterinary Association. Veterinary continuing education publication No. 175. Palmerston North, Massey University.

Mitchell, R.L.; Ruth, J.W.S.; Johnson, I.M. 1957. Trace element uptake in relation to soil content. *Journal of the science of food and agriculture 8*: 51–59.

New Zealand Food Regulations 1984. Regulation #257. Wellington, Ministry of Health.

SAS Institute 1987. SAS User's Guide: Statistics. Cary, North Carolina, USA, SAS Institute, Inc.

Sherrell, C.G.; Rawnsley, J.S. 1982. Effect of copper application on copper concentration in white clover and perennial ryegrass on some northland soils and a yellow-brown pumice soil. *New Zealand journal of agricultural research 25*: 363–368.

Sykes, A.R.; Nicol, A.M. 1983. The energy requirements of the weaned lamb. pp. 121–134. *In:* Lamb Growth. Familton, A.R. (ed.) Animal Industries Workshop, Lincoln College.

Todd, J.R. 1969. Chronic copper toxicity of ruminants. *Proceedings of the Nutrition Society 28*: 189–198.

West, D.M.; Vermunt, J.J; Sargison, N.D. 1997. Copper supplementation of beef bulls – Benefits can be measured. pp. 39–46. *In:* Proceedings from the 27th Seminar of the Society of Sheep and Beef Cattle Veterinarians. New Zealand Veterinary Association. Veterinary continuing education publication No. 175. Palmerston North, Massey University.

Woolliams, J.A.; Suttle, N.F.; Wiener, G.; Field, A.C.; Woolliams, C. 1982. The effect of breed of sire on the accumulation of copper in lambs, with particular reference to copper toxicity. *Animal production 35*: 299–307.

Woolliams, J.A.; Suttle, N.F.; Wiener, G.; Field, A.C.; Woolliams, C. 1983. The long-term accumulation and depletion of copper in the liver of different breeds of sheep fed diets of differing copper content. *Journal of agricultural science, Cambridge 100*: 441–449.

Woolliams, J.A.; Wiener, G.; Woolliams, C.; Suttle, W.F. 1985. Retention of copper in the liver of sheep genetically selected for high and low concentrations of copper in plasma. *Animal production 41*: 219–226.

■