Variation in copper metabolism between two flocks of Romney sheep in response to increasing dietary copper

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ABSTRACT

Differences in the Cu status of two flocks of Romney lambs were observed which suggest that lines of sheep having distinct Cu metabolism have been identified. As part of a larger study relating Cu intake to Cu status of grazing lambs, changes in concentrations of Cu in liver, plasma and other tissues, and in erythrocyte Cu/Zn superoxide dismutase (SOD) were monitored for 176 days in two flocks of 50 sheep. The animals grazed on ten experimental paddocks treated with 0, 0.4 or 4 kg Cu/ha which resulted in mean pasture herbage Cu concentrations of 8 (Control), 13 (Low), and 41 (High) mg Cu/kg DM. At the start of the experiment these lambs, sourced from two Wairoa farms, had markedly different liver Cu reserves (1270 v 190 µmol Cu/kg FW, flocks H and L respectively) which could not be attributed to Cu supplementation. This difference between flocks was maintained throughout the 176 day study for sheep grazed on control pastures, through day 99 for sheep on the low pastures, and for 51 days in sheep on the high paddocks. High copper intakes increased liver Cu concentrations to as much as 4800 µmol (300 mg) Cu/kg liver FW after 99 days, with no signs of chronic Cu toxicity. Initial flock differences in liver Mo concentration (14.7 v 20.6 µmol Mo/kg FW, n=10, $P<0.05$) disappeared by day 51. The first measured SOD activity (day 51) in flock H was 17% greater than that of flock L (1600 v 1370 U/g Hb, n=21, $P<0.01$) and this flock difference was maintained through day 142 and day 99 for sheep grazing control and low pastures, respectively. SOD activity was found to be a reasonable predictor of liver Cu stores only in sheep having low to moderate Cu intake. Had the differences in initial liver Cu concentration and in blood Cu/Zn SOD activity of the two flocks been merely a reflection of the Cu and Mo status of their respective Wairoa farms, the disparity would have been expected to diminish much more quickly than was observed in this study. Factors controlling Cu metabolism are heritable. Trials selecting for differences between flocks in liver Cu concentration are needed to establish whether these flocks are genetically distinct in the way they metabolise Cu.

Keywords: heritability; liver Cu; erythrocyte Cu/Zn SOD; pasture Cu; Mo.

INTRODUCTION

Genetic diversity in Cu metabolism among UK breeds of sheep such as Scottish Blackface and Welsh Mountain has been described. Efficiency of Cu absorption and utilisation (Suttle, 1974), plasma Cu concentrations, and blood SOD activities (Weiner *et al*., 1985) have all been shown to have some degree of heritability. These traits can affect liver Cu accumulation (Woolliams *et al*., 1985), sensitivity to deficient dietary Cu and susceptibility to Cu toxicity (Suttle, 1977), and can impact on adaptation to changing Cu intake. Among traditional New Zealand breeds of sheep, for example Romney, Perendale, Coopworth, genotypic variability of Cu metabolism between and within breeds has yet to be demonstrated.

In this study, two flocks of Romney sheep identified as having different initial liver Cu concentrations were grazed on Cu fertiliser treated and untreated pastures for up to six months and were monitored for evidence of difference in Cu metabolism.

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*., 1997), pastures were topdressed with various amounts of copper sulphate. The $CuSO_4 \cdot 5H_2O$ was thoroughly mixed with superphosphate fertiliser and the copper-amended fertiliser applied by hand in mid April to ten experimental paddocks at the rate of 250 kg/ha. This was designated as day 1 of the study. The treatments were superphosphate with no additional Cu (Control), superphosphate plus 0.4 kg Cu/ha (Low), superphosphate plus 4.0 kg Cu/ha (High), on three, three and four paddocks respectively. After 30 days, mean Cu concentration of herbage sampled from the blocks of treated paddocks was 8.2, 17.8 and 30.3 mg Cu/kg DM, respectively. Mean pasture Cu concentrations for the trial period, calculated for days 30 through 141, were 8 ± 0.5, 13 ± 1.3 and 41 ± 3.6 mg Cu/kg DM.

A survey by Korte *et al*., (1997) of the Cu status of beef and sheep farms in the east coast region of the North Island, New Zealand (Wairoa and Gisborne areas) showed that, on a number of farms where all the cattle were Cu deficient (i.e., liver Cu concentrations <95 µmol Cu/kg fresh weight FW), the sheep grazing those farms could be classified as having either adequate to high, or low Cu status. The two flocks of eight month old Romney lambs used in this study were sourced from two Wairoa farms: Lambs from Frasertown had high Cu status (flock H, 1270 µmol Cu/kg liver FW), and lambs from Raupunga had low Cu status (flock L, 190 µmol Cu/kg liver FW), and this difference was not attributable to Cu supplementation.

Pasture composition of the two Wairoa farms contained 9.0 and 5.7 mg Cu/kg FW, and 1.1 and 3.3 mg Mo/kg FW, respectively ($P<0.05$).

The lambs were transported to AgResearch Ballantrae Research Station, near Woodville, and for four weeks prior to the experiment were grazed as a single group. In early May (17 days after fertiliser application) the lambs were eartagged, weighed, randomly assigned to experimental paddocks, and set stocked at 12 animals/ha on paddocks of average area 0.79 ha. Liveweights were recorded at days 1, 50, 141 and 175 of the study. Two lambs from each paddock were designated as monitor animals and liver biopsy samples were obtained from these at day 1 and again at days 51, 99 and 142. Blood was collected from every lamb at days 17, 51, 99, 142 and 175. At day 176 all lambs were slaughtered at a local abattoir.

Samples collection

Pasture 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 prior to analysis for Cu and other elements.

Blood Four to six ml 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 the erythrocytes washed twice with cold 0.9% NaCl. Plasma and erythrocytes were stored at -20°C prior to analysis of Cu and Cu/Zn superoxide dismutase (SOD) activity.

Liver Samples of 50 to 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. At the time of slaughter, the entire liver was collected, washed in cold 0.9% NaCl, and stored at -85°C.

Analytical

Elemental composition of pasture and liver 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 2M HCl. Copper in plasma was determined by ICP after digesting 1 ml samples in 0.5 ml concentrated Analar HNO_3 and 0.5 ml 30% H_2O_2 in polyethylene tubes in a waterbath for two hours at 90°C. The Cu/Zn SOD activity of lysed erythrocytes was determined spectrophotometrically using a kit supplied by Randox Limited (Crumlin, Northern Ireland) based on the method of Podczasy and Wei (1988).

Statistical analyses included t-test, linear regression, ANOVA and Duncan's multiple-range test, using procedures of Statistical Analysis System (SAS, 1985). Where appropriate, data collected at intervals throughout the trial were analysed by repeated measures with time (trial day) as the factor. Liver Cu concentration data were subjected to $\log_{10}$ transformation prior to statistical analysis due to heterogeneity of variance among treatments. Except where otherwise noted, level of probability is P - 0.05.

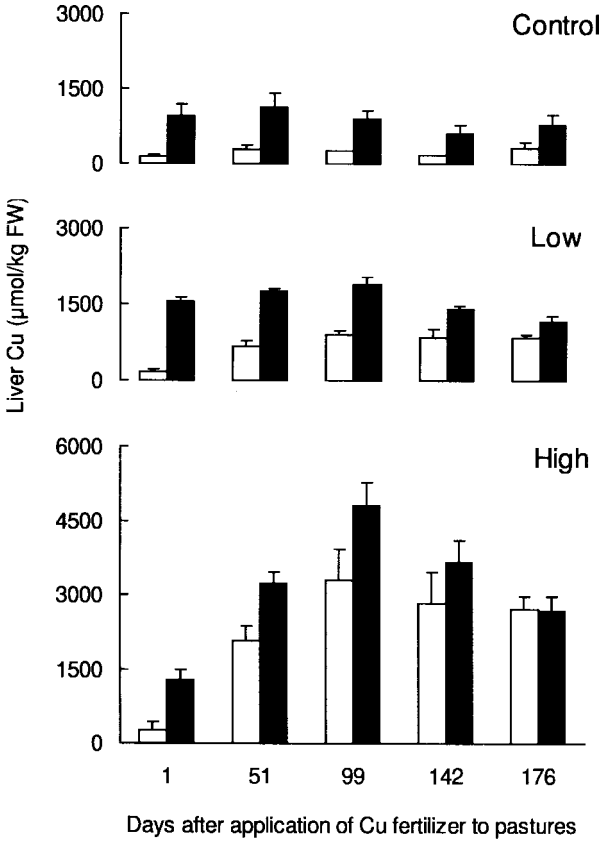
RESULTS AND DISCUSSION

Topdressing significantly increased pasture Cu concentrations by 30 days after application, with maximal concentrations of Cu between 30 and 65 days. By day 374 Cu levels had returned to baseline. Treatments applied to the ten paddocks did not significantly affect pasture concentration (mean ± standard error) of most other elements, including Ca 3.4 ± 0.06, K 32.4 ± 0.88, Mg 2.1 ± 0.02, Na 1.0 ± 0.03, P 4.6 ± 0.08 and S 3.3 ± 0.05 g/kg DM; and Fe 459 ± 43, Mn 396 ± 17, Mo 1.0 ± 0.04, Zn 36.9 ± 0.08 mg/kg DM.

The mean initial (day 1) liveweight of all lambs was approximately 31 kg. Neither flock or treatments affected average weight gain (90 ± 2.4 g/day), body weight at slaughter (46.8 ± 0.43 kg, n=92), or final liver weight (0.84 ± 0.008 kg, n=82).

The effects of increasing pasture Cu concentrations on the liver Cu concentrations of flock L and flock H during the 176 day study are shown in Figure 1. High copper intakes increased liver Cu concentrations to as much as 4800 µmol (300 mg) Cu/kg liver FW, with no signs of chronic Cu toxicity. The initial (day 1) liver Cu concentrations of flock H were six times greater than those of flock L (1270 v 190 µmol Cu/kg FW, n=10, $P<0.001$), although the Cu content of pastures on their Wairoa source

FIGURE 1: Differences in the liver Cu concentrations of two Romney flocks (□ flock L; ■ flock H) in response to increasing pasture Cu concentrations. Control (8 mg Cu/kg DM), Low (13 mg Cu/kg DM), High (41 mg Cu/kg DM). Data are from liver biopsies of monitor animals (days 1 to 142, average n=3) and from samples of liver collected at slaughter (day 176, n=14). Mean ± SEM.



farms differed only 1.6-fold. A significant difference between flocks was maintained throughout the 176 day trial for sheep grazed on control pastures, but only through days 99 and 51 for sheep on low and high Cu pastures, respectively. The difference remained for at least six months of grazing pastures of adequate Cu content, suggesting that the substantially larger Cu pool size in liver of flock H was being maintained by greater Cu absorption, lesser endogenous Cu loss, or both. That increasing dietary Cu caused the flock difference to diminish over time in a Cu dose response indicates that any flock distinctions in these Cu metabolic processes are probably saturated at high Cu intakes.

Erythrocyte Cu/Zn SOD activity provides another measure of animal Cu status. The effect of increasing pasture Cu concentration on erythrocyte Cu/Zn SOD activity in the two flocks over the 176 day study is shown in Figure 2. Among all sheep the first measured activity (day 51) of flock H was 20% greater than that of flock L (1590 v 1330 U/g Hb, n=18, P<0.01). This difference remained through day 142 and day 99 for flocks grazing the control and low pastures, respectively. Activity was increased by increasing Cu intake (P<0.001), with interactions by time (P<0.01) and flock (P<0.05). Liver Cu concentration was found to be a weak predictor of Cu/Zn SOD activity (ln transformed data; $R^2 = 0.43$, n=93, P<0.001). The greater activity of this Cu-dependent enzyme in flock H during four months of grazing suggests that at least some of that flock's larger liver Cu stores are not simply sequestered but represent metabolically available Cu.

The liver Mo concentrations of flock H were initially less than those of flock L (14.7 v 20.6 µmol Mo/kg FW, n=10, P<0.05), as would be expected based on the Mo content of the Wairoa farm pastures, but this flock difference disappeared by day 51. Data for day 1 and day 176 are presented in Table 1. Grazing the low Mo pastures at Ballantrae station (1.0 ± 0.04 mg Mo/kg DM) reduced overall mean liver Mo concentrations at day 176 compared to day 1 (11.2 v 17.6 µmol Mo/kg FW, n=89 and 20, P<0.001). Increasing mean pasture Cu concentration from 8 to 41 mg Cu kg^{-1} DM resulted in decreased liver Mo concentration (P<0.001), possibly due to Cu-Mo-S interaction. The difference in initial liver Mo concentration between the two flocks was rapidly equalised by low Mo intake on Ballantrae pastures, in contrast to the persistence of differences of Cu status between flocks grazing control pastures.

FIGURE 2: Differences in the erythrocyte Cu/Zn SOD activity of two Romney flocks (□ flock L; ■ flock H) in response to increasing pasture Cu concentrations. Control (8 mg Cu/kg DM), Low (13 mg Cu/kg DM), High (41 mg Cu/kg DM). Mean ± SEM, n=6.

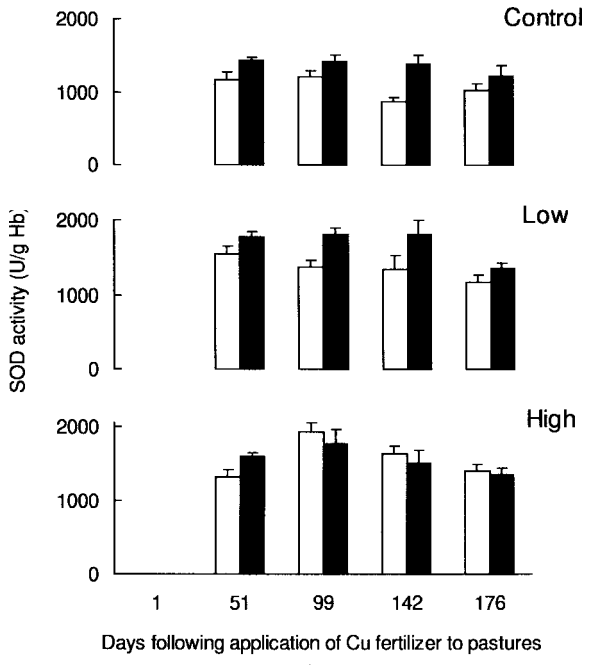


TABLE 1: Differences in the liver Mo concentration of two Romney flocks in response to increasing pasture Cu concentrations. Data are from liver biopsies taken at the start of the trial (day 1), and from livers collected at slaughter (day 176). Mean ± SEM.

			Flock L	Flock H
Pasture Cu concentration[1]	day	n	µmol Mo / kg liver FW2	
(pre-experimental)	1	10	20.6 ± 2.26	14.7 ± 0.85*
Control	176	12	13.3 ± 0.67[a]	12.4 ± 0.81[a]
Low	176	15	11.4 ± 0.68[b]	10.9 ± 0.46[ab]
High	176	17	9.7 ± 0.56[b]	10.2 ± 0.48[b]

[1] Control 8 mg Cu/kg DM; Low 13 mg Cu/kg DM; High 41 mg Cu/kg DM.
[2] Day 176 means with different superscript differ significantly (P<0.05).
Asterisks indicate significant *P* value by t-test between flocks (P<0.05).

CONCLUSIONS

In this study we observed differences in how Cu was stored and utilised by two flocks of Romney sheep which persisted through months of grazing Cu treated and untreated pastures. Liver Cu concentration and erythrocyte Cu/Zn SOD activity were typically greater in flock H compared to flock L. This might be explained by genetic variation in the mechanisms of Cu absorption and excretion. Had the differences in initial liver Cu concentration and in SOD activity of the two flocks been merely a reflection of the Cu and Mo status of their respective Wairoa farms, the disparity would have been expected to diminish much more quickly. Heritability trials selecting for differences between flocks in liver Cu concentration are needed to establish whether these flocks are genetically distinct in the way they metabolise Cu.

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