Effect of increasing elemental sulphur and copper intakes on the copper status of grazing sheep

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Abstract

Under some conditions, the use of high-sulphur fertilisers has been thought to be associated with an increase in the incidence of copper deficiency among ruminants grazing the fertilised pastures or fed treated herbage. In this study 35 Romney lambs grazing ryegrass–white clover pasture of low molybdenum content (<0.5 mg Mo/kg DM) received dietary supplements of elemental S and Cu 3 times weekly for 15 weeks. The supplements increased S intake from 3.9 to 7.9 g/day, and increased Cu intake from 9.3 to 24.3 mg Cu/day. Treatment with S significantly increased the total S concentration in strained rumen fluid (SRF), and treatment with Cu increased the Cu concentrations in all fractions of the digesta. In the presence of 4 g S/day, the effect of supplemental Cu on SRF and duodenum soluble Cu concentrations was reduced by about half. Increasing S intake had no effect on plasma and liver Cu concentrations, while increasing Cu intake markedly raised liver Cu concentration. It was concluded that fertilising pastures with elemental S is unlikely to affect the Cu status of grazing sheep, under conditions where pasture Mo concentration is low (<0.5 mg Mo/kg DM).

Keywords: copper, fertiliser, liver, plasma, Romney, sulphur

Introduction

Awareness of phosphorous (P) and sulphur (S) deficiencies in some New Zealand soils has led to increased use of S fertilisers containing a high proportion of elemental S (During 1984), typically applied at 30 to 90 kg S/ha. Under some conditions, the application of high-S fertilisers has been thought to be associated with an increase in the incidence of copper (Cu) deficiency among ruminants grazing the fertilised pastures or fed treated herbage. Dietary elemental S and sulphate is reduced to sulphides in the reticulorumen (Hale & Garrigus 1953) and then reacts with dietary Cu to form insoluble CuS, or with Cu and molybdenum to form insoluble Cu thiomolybdates, which can interfere with the absorption and storage of Cu (Bird 1970; Mason 1982). For instance, a 50% decrease in the apparent absorption of dietary Cu was reported when lambs were fed silage made from sorghum fertilised with 138 kg S/ha as ammonium sulphate (Ahmad *et al.* 1995). Dietary Cu deficiency owing to low Cu intake or diminished Cu absorption leads to depleted liver Cu stores and lowered blood plasma Cu concentration (Grace 1994).

This study describes changes in the S and Cu metabolism of Romney sheep given supplemental elemental S and Cu, via monitoring concentrations of Cu in liver and plasma, and concentrations of S and Cu in reticulorumen, abomasum and duodenum digesta.

Materials and methods

This experiment was part of a larger investigation of the relationship between changing dietary S and Cu intakes and the Cu status of grazing lambs (Grace *et al.* 1997). Experimental design was based on an incomplete factorial arrangement of supplemental elemental S (0, 2 or 4 g elemental S/day) and supplemental Cu (0 or 15 mg Cu/day as $CuSO_4.5H_2O$). The five treatments were: 0 S + 0 Cu (Control); 2 g S + 0 Cu; 4 g S + 0 Cu; 0 S + 15 mg Cu; and 4 g S + 15 mg Cu.

Thirty-five castrated Romney lambs weighing 34 to 35 kg each were randomly assigned to five treatment groups of seven animals. The animals were eartagged and grazed as a single flock on ryegrass–white clover pasture at Aorangi Research Station, near Palmerston North. Three times each week for 15 weeks the sheep were mustered to receive S and Cu supplements, which were weighed into gelatine capsules and administered by baling gun to the reticulorumen. At monthly intervals the sheep were weighed and blood samples collected.

Collection and analysis of samples

Herbage samples (»400 g fresh pasture) were collected each month along the same 100–150 m transect 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. At the end of the study the animals were killed using a captive bolt gun. At that time livers were removed and weighed, and samples of digesta were collected from the reticulorumen, abomasum and duodenum. Digesta was strained of large particles and centrifuged at 30 000 × g for 40 minutes and the supernatant fraction collected for analysis of "soluble" Cu (Lee 1989).

Elemental composition of pasture, liver, plasma and digesta supernatants was determined by inductively coupled plasma (ICP) emission spectrometry (Lee 1983). In preparation for ICP analysis, 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.

Significant differences among treatment means were determined by ANOVA and means separated by LSD, using individual animals as replicates.

Results

Pasture elemental composition was (mean ± SEM): 2.6 ± 0.22 g S, 1417 ± 250 mg Fe, 6.2 ± 0.4 mg Cu, 37 ± 3.1 mg Zn and <0.5 mg Mo/kg DM, with little seasonal variation. Treatments did not affect animal growth; average daily weight gain was 90 ± 10.7 g/day. Based on pasture S and Cu concentrations and an estimated daily DM intake of 1.5 kg/day for 40 kg lambs gaining 90 g/day (Geenty & Rattray 1987), dietary intake of S and Cu derived from pasture alone was calculated to be 3.9 g S/day and 9.3 g Cu/day.

Supplemental S (2 or 4 g S/day) had no effect on plasma and liver Cu concentrations (Table 1). Supplemental Cu (15 mg Cu/day) did not change plasma Cu concentrations, but significantly increased ($P<0.01$) liver Cu concentration. There were no significant interactions of supplemental S and Cu on plasma and liver Cu concentrations.

Table 1 Effects of increasing dietary intake of elemental S and Cu on Cu concentrations in liver and plasma of grazing Romney sheep (mean ± SEM, n = 7).

---- Treatment[1] ----		------- Cu concentration[2] -------	
S g/day	Cu mg/day	Plasma mg/l	Liver mg/kg fresh wt.
0	0	0.85 ± 0.11	43 ± 15[A]
2	0	0.83 ± 0.03	66 ± 15[A]
4	0	0.90 ± 0.14	69 ± 15[A]
0	15	0.84 ± 0.05	168 ± 37[B]
4	15	0.87 ± 0.05	175 ± 19[B]

[1] Treatments are supplemental to the dietary intake from pasture: 3.9 g S/day, 9.3 mg Cu/day.
[2] Within-column means with different superscripts are significantly different: A vs B, P<0.01.

Supplemental S significantly increased ($P<0.05$) the total S concentration in strained rumen fluid (SRF), and this response was not affected by supplemental Cu (Table 2). Supplemental Cu markedly increased the Cu concentrations in all fractions of the digesta. In the presence of 4 g S/day, this effect of supplemental Cu was reduced by about half.

Discussion

The metabolism of S in ruminants is complex. All sources of dietary S are reduced to sulphide in the reticulorumen (Lewis 1954) before being converted to microbial protein or absorbed as H_2S (Bray & Till 1975). In sheep fed fresh pasture 10–15% of total S in the reticulorumen is in the form of sulphides, with the remainder as organic S (Lee 1989).

In this study, pasture alone supplied 3.9 g S/day, and S supplements increased dietary intake to as much as 7.9 g S/day. High S intake can be toxic. For instance, diets providing 9.5 g S/day (6.3 g S/kg DM) have caused polioencephalomalacia in sheep (Olkowski & Gooneratne 1992), a disorder characterised by focal necrosis of grey matter in the brain. In this study, Romney lambs receiving 7.9 g S/day for 15 weeks

Table 2 Effects of increasing dietary intake of elemental S and Cu on the S and Cu concentrations in fractions of digesta from reticulorumen, abomasum and duodenum of grazing Romney sheep (mean ± SEM, n = 7).

------ Treatment[1] ------		---------------- Reticulorumen ---------------- strained rumen fluid		Reticulorumen	Abomasum	Duodenum
				30 000 × g supernatant		
S g/day	**Cu** mg/day	**total S** mg/g DM	**soluble Cu** µg/l	**soluble Cu** µg/l		
0	0	4.07 ± 0.65[a]	420 ± 53[AB]	74 ± 9[A]	31 ± 8	115 ± 18[a]
2	0	5.85 ± 0.43[b]	500 ± 46[B]	76 ± 14[A]	28 ± 9	141 ± 19[a]
4	0	7.69 ± 0.89[c]	350 ± 30[A]	61 ± 6[A]	31 ± 9	64 ± 10[b]
0	15	3.40 ± 0.14[a]	5340 ± 1623[D]	439 ± 179[B]	23 ± 13	472 ± 243[c]
4	15	8.38 ± 0.93[c]	2510 ± 343[C]	303 ± 108[B]	72 ± 38	280 ± 102[c]

[1] Treatments are supplemental to the dietary intake from pasture: 3.9 g S/day, 9.3 mg Cu/day.
[2] Within-column means with different superscripts are significantly different: a vs b, P<0.05; A vs B, P<0.01.

showed no clinical signs of S toxicity, and their average weight gain was 90 g/day.

When Cu intake and absorption exceed metabolic demands of the tissues, the excess Cu accumulates in liver, the site of 50–60% of total body Cu. Thus, changes in liver Cu concentration are the most reliable indicator of the Cu status of sheep (Grace *et al.* 1998). Liver Cu is stored in association with metallothionein, incorporated into the transport protein caeruloplasmin, or excreted in bile. When Cu supplements increased dietary intake from 9.3 to 24.3 mg Cu/day, liver Cu concentrations were raised three-fold, regardless of the presence or absence of supplemental S (Table 1). In contrast, a study by Dick (1954) reported that increasing dietary sulphate from 0.04 to 2.4 g S/kg DM reduced by 26–30% the concentration Cu in liver. Bird (1970) showed that increasing dietary S as sulphate or S-amino acids from 0.78 to 2.48 g/kg DM reduced by half the flow of soluble Cu to the small intestine. Suttle (1974) monitored Cu concentrations in plasma from Cu depleted and repleted sheep and found that increasing S intake as sulphate or methionine from 1 to 4 g S/kg DM decreased the absorption coefficient of Cu from 0.062 to 0.041. This S × Cu interaction might be explained by high concentrations of free S^{2-} in the digestive tract of S-supplemented animals, with the subsequent formation of CuS, an insoluble compound which is not absorbed but excreted in the faeces (Suttle 1974).

The impact of supplemental S on Cu absorption and metabolism is greater with low S diets than high S diets. Increasing S from 0.5 to 2.0 g/kg DM diminishes Cu absorption more than does increasing S from 3 to 5 g/kg DM (Suttle & McLauchlin 1976). Sulphur content of the basal diet (pasture contribution) in this study was about 2.6 g S/kg DM, a level at which the influence of additional S on Cu metabolism is likely to be small. Supplementation with up to 4 g S/day increased dietary intake to 5.2 g S/kg DM, a level substantially higher than that of the diets used by Dick, Bird, and Suttle (above).

The source as well as the level of dietary S can affect S × Cu interaction. When elemental S is applied as a fertiliser, it is washed into the soil, slowly oxidised to sulphate and taken up by the plant, and results in only small changes in herbage S concentration. Thus S fertilisers are unlikely to substantially increase S intake of grazing ruminants, unless the animals ingest large amounts of S-contaminated soil. In New Zealand, where S concentrations of grazed pastures typically range from 2.5 to 4 g S/kg DM (Grace 1983), this additional S intake is unlikely to affect Cu absorption and accumulation.

The impact of Mo × S on Cu metabolism is greater than that of S alone. Dietary Mo intake greater than 2 mg Mo/kg DM in the presence of S markedly reduces Cu absorption and storage through the formation of Cu-thiomolybdates in the digestive tract and their subsequent interference with utilisation of Cu in tissues, especially the liver (Suttle 1975; Mason 1982). In this study Mo intake of <0.5 mg Mo/kg DM was probably too low for Mo × S interactions to have measurable effects on plasma and liver Cu concentrations (Lamand 1989). Increasing S intake from 3.9 to 7.9 g S/day did reduce by about half the Cu concentrations in SRF and in duodenum digesta supernatant of the low-Cu sheep (Table 2), but clearly adequate amounts of Cu were still absorbed from the intestinal region, as plasma and liver Cu concentrations were maintained (Table 1).

This study concludes that use of high S fertilisers is unlikely to increase the incidence of Cu deficiency in sheep.

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