Copper deficiency in ruminants in the South East of South Australia

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Summary. Pasture development in the South East of South Australia has depended upon trace element enriched fertiliser applications. Despite the wide usage of copper-enriched fertilisers, copper deficiency is still evident in livestock at pasture, particularly cattle.

Serum collected from cows and heifers during the systematic sampling program of the Brucellosis and Tuberculosis Eradication Scheme was analysed for copper. Of the 3611 pooled herd samples analysed, approximately 9% had low serum copper concentrations (<7 µmol/L). Distribution of those herds identified to be at risk of copper deficiency appeared to be random, apart from areas of high risk on peat soils and the coastal fringe of calcareous sands.

Introduction

The successful development of pastures on soils in the South East of South Australia has relied on copper (Cu) and zinc (Zn) enriched fertilisers to sustain pasture and livestock production (Riceman and Anderson 1943; Marston and Lee 1948; Riceman 1948; Tiver 1955). Riceman (1961) concluded that, if these trace elements have been applied at the recommended rate at the time of land clearing and pasture establishment, only single superphosphate is needed to maintain Cu and Zn levels for pasture production. Reuter (1975) reported that Riceman based this conclusion on observations that pasture and livestock production were maintained for at least 7 years after the initial application, as applied Cu is not readily leached (Jones and Belling 1967); and the Cu and Zn removed in farm produce approximately equals their addition as impurities in annual dressings of superphosphate.

Hannam et al. (1982) concluded that a Cu dressing of 2 kg Cu/ha to the sandy soils of the Upper South East of South Australia provided adequate Cu for pasture and sheep production for at least 23 years. However, Hannam and Reuter (1977) were able to identify 26 properties in the South East that produced 'steely' wool between 1972 and 1975. 'Steely' wool being an early and specific indication of Cu deficiency (Marston and Lee 1948). Hannam and Reuter (1977) also noted that steely wool is more common in wetter years and is rarely reported in drier seasons. The Cu economy of cattle appears to be inferior to that of sheep (Roberts 1976), and Cu deficiency in cattle has occurred widely on pastures developed with Cu fertiliser (Cunningham 1979).

The Brucellosis and Tuberculosis Eradication Scheme provided the opportunity for surveying the mineral nutrition of beef and dairy cows by analysis of blood samples collected primarily for brucellosis screening. In the present study, 3611 herd samples collected between April 1979 and April 1981 were analysed for serum copper. The sampling area represented all the South East which sustains 30% of the sheep and 50% of the cattle in South Australia.

With pasture sampling to complement the serum analysis, the aim of the survey was to determine the extent, distribution and likely cause of the Cu deficiency in grazing livestock.

Materials and methods

Blood samples

Staff appointed at 5 centres in the South East under the Brucellosis Eradication Scheme and local private veterinary practitioners collected blood samples

Analysis of pasture samples collected from paddocks with cattle having low serum copper concentrations showed that low serum copper was usually associated with raised molybdenum rather than low copper concentrations in pasture. In some instances, moderate concentrations of molybdenum and sulfur in pasture and soil ingestion associated with high iron concentrations may combine to cause hypocupraemia, especially when livestock graze stubbles and subterranean clover pastures in summer-autumn and short pastures in winter.

Only 6% of pasture samples had less than 4 mg Cu/kg DM, a concentration which indicates possible copper deficiency in subterranean clover or strawberry clover.
systematically from all beef and dairy cows and heifers on individual properties. The survey was conducted from April 1979 to April 1981 and included 3611 herd samples. These samples accounted for 65% of the 2991 registered herds in the region. Resampling of some herds took place where brucellosis was suspected and in the normal retesting program. Samples were collected from the coccygeal vein into evacuated glass tubes. Random samples of serum from 8–10 tubes (representing 8–10 cows or heifers) from each herd were pooled on an approximately equal volume basis for Cu analysis. Serum was deproteinised with 10% (w/v) trichloroacetic acid and Cu assayed in the supernatant (Judson et al. 1982). Serum Cu concentrations of herds were related back to their origin of County, Hundred and Section to determine their geographical distribution.

There was no differentiation made between beef or dairy cows and heifers in the survey. No allowance was made for any Cu supplements provided to the cattle, of which 30% receive some form of supplement (C. L. Trengove, unpublished data). As sampling continued throughout the year, hand feeding would have been undertaken in most herds; however, hay cut on the property is the usual feed supplement. No allowance was made for the possible recent introduction of cattle onto particular properties.

Pasture samples

Pasture samples were initially collected in response to the identification of herds with low serum Cu concentrations where the owner had the recorded Cu fertiliser history of the property. Subsequently, pastures on other properties were sampled from areas where no Cu deficiency was indicated. The amount of Cu applied and the date of the latest application was related to Cu concentration in the pasture. Overall, the 97 sites sampled gave a good representation of the major soil types of the region.

Initially, 10 samples per paddock were collected but this was later reduced to 5 samples. Pastures were sampled during July–September. Each sample consisted of at least 30 subterranean clover or strawberry clover runners (or plants; depending on growth stage). Clover was sampled as it forms the basis of the pastures in the region. Samples were collected into rice paper bags. The youngest open leaf tissue was dissected from the sample and analysed for Cu to determine if Cu deficiency affected clover growth (Reuter et al. 1981). The remainder of each sample was dried at 60°C and ground in a stainless steel mill prior to analysis for Cu, molybdenum (Mo), sulfur (S) and iron (Fe) concentrations in dry matter (DM), as these elements have been shown to be important in determining Cu availability in ruminants (Dick 1956; Campbell et al. 1974; Suttle et al. 1975; Humphries et al. 1983).

Copper and Fe were analysed by atomic absorption spectrophotometry following nitric–perchloric acid digestion of the samples. Total S was analysed by X-ray fluorescence spectrophotometry and Mo was analysed colorimetrically following nitric–perchloric and sulfuric acid digestion of samples (modified from Bingley 1963).

Data have also been included on Mo and S concentrations from pasture sampled during a soil acidity survey (Hodge and Lewis 1989). A single sample of clover (whole tops) was collected randomly throughout each of the paddocks identified as having soil (0–10 cm depth) with a pH <5.5 (1:5, soil:water).

One-way analysis of variance was used to determine significance (P<0.05) of the individual soil types on the Cu, Mo, S and Fe concentrations of the pasture.

Results and discussion

Serum copper concentrations

With a serum Cu concentration of <7 μmol/L considered to indicate a low Cu status, the survey showed that at least 9% of herds in the region could be at risk to Cu deficiency (Table 1). In using serum Cu rather than plasma Cu we may have overestimated the number of herds low in Cu (Paynter 1982), but in using mean values we may have missed herds in the early stage of Cu depletion. Other herds may be at risk owing to the serum concentration being a mean of sera from a number of animals (8–10). Individuals with high serum concentrations may mask the animals with low serum Cu.

Hypocuprosis is often diagnosed in a portion of a herd (Jarvis and Austin 1983). The differences between animals in their susceptibility to copper deficiency is most likely due to differences in their capacity to build up Cu reserves or in their ability to utilise Cu available in diets of low Cu availability (Jarvis and Austin 1983).

<table>
<thead>
<tr>
<th>County</th>
<th>No. of herd samples</th>
<th>Copper concentration (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;5</td>
<td>≤7</td>
</tr>
<tr>
<td>Hundreds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grey (21)</td>
<td>1392</td>
<td>28 (2.0)</td>
</tr>
<tr>
<td>Robe (18)</td>
<td>1010</td>
<td>6 (0.6)</td>
</tr>
<tr>
<td>MacDonnell (15)</td>
<td>585</td>
<td>4 (0.7)</td>
</tr>
<tr>
<td>Cardwell (12)</td>
<td>355</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td>Buckingham (11)</td>
<td>269</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Total</td>
<td>3611</td>
<td>42</td>
</tr>
<tr>
<td>% of Total</td>
<td>1.2</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Table 1. The number of herds (cumulative totals) with serum copper concentrations of <5, ≤7, ≤8, >8 μmol/L sampled in Counties of South East of South Australia

The percentage of total number of herds for each copper concentration is in parentheses.
Copper deficiency in ruminants (Lee 1951). These Hundreds also contain significant areas of peat soils on which grazing animals are inherently Cu deficient (Deland et al. 1986; Gartrell 1981).

Similarly in County Robe, the Hundred of Waterhouse, adjoining the coast (Fig. 1), has the highest incidence of low Cu concentrations of the County (Table 2). Despite the awareness of Cu deficiency in these areas, it appears that suitable supplements are not being provided to many of the herds to maintain adequate Cu status.

Low serum Cu concentrations (Table 2) also occurred in the inland Hundreds of Riddoch, Jessie and Binnum (Fig. 1) and peat soils do not occur in Jessie and Binnum. The soil types within these Hundreds are dominated by acidic red gum soils (solodised solonetz, Dy 5; after Northcote 1979) which are prone to waterlogging. The incidence of low serum Cu concentrations appears to be at random on this soil type.

The areas where there was a low incidence of low Cu deficiency were Rivoli Bay, Lake George and MacDonnell (Table 2), all of which adjoin the coast (Fig. 1). Coast disease of sheep and cattle, a dual cobalt (Co) and Cu deficiency, has historically been a problem.

**Table 2. The number of herds (cumulative totals) with serum copper concentrations of <5, ≤7, ≤8, >8 μmol/L sampled within selected Hundreds of Counties Grey, Robe and MacDonnell, in the South East of South Australia**

The percentage of total number of herds is in parentheses.
Table 3. The mean and range (in parentheses) of copper, molybdenum, sulfur and iron concentrations in subterranean and/or strawberry clover whole tops sampled from the major soil types of sand/clay (ground water podsol), red gum (solodised solonetz and solon), ground water rendzina, deep sand (podsol), alkaline peat and calcareous sand, which occur in the South East of South Australia

Mean values each comprised of 5-10 samples. For each mineral, mean concentrations followed by different letters differ significantly (Pc0.05)

<table>
<thead>
<tr>
<th>Soil type</th>
<th>No. of sites</th>
<th>No. of samplings</th>
<th>Cu (mg/kg)</th>
<th>Mo (mg/kg)</th>
<th>S (g/kg)</th>
<th>Fe (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand/clay</td>
<td>24</td>
<td>41</td>
<td>6.6a (3.0-14.6)</td>
<td>1.4a (0.1-4.1)</td>
<td>2.5a (1.5-4.0)</td>
<td>160a (45-346)</td>
</tr>
<tr>
<td>Red gum</td>
<td>24</td>
<td>86</td>
<td>9.5b (4.1-15.9)</td>
<td>1.8a (0.1-5.4)</td>
<td>2.6a (1.5-4.3)</td>
<td>520b (81-2300)</td>
</tr>
<tr>
<td>Ground water rendzina</td>
<td>20</td>
<td>34</td>
<td>8.5ab (4.2-12.8)</td>
<td>1.6a (0.2-5.4)</td>
<td>2.5a (1.9-5.1)</td>
<td>510b (200-1000)</td>
</tr>
<tr>
<td>Deep sand</td>
<td>16</td>
<td>22</td>
<td>7.3a (4.0-14.0)</td>
<td>1.1a (0.1-3.8)</td>
<td>2.8a (2.1-4.6)</td>
<td>140a (119-154)</td>
</tr>
<tr>
<td>Peat</td>
<td>4</td>
<td>7</td>
<td>7.2a (4.4-11.4)</td>
<td>8.3b (4.7-16.2)</td>
<td>3.6b (2.8-4.5)</td>
<td>110a (48-320)</td>
</tr>
<tr>
<td>Calcareous sand</td>
<td>11</td>
<td>23</td>
<td>5.3a (1.9-9.5)</td>
<td>10.1b (1.6-21.8)</td>
<td>2.5a (1.7-3.4)</td>
<td>100a (70-130)</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>213</td>
<td>2.5a (1.5-4.0)</td>
<td>2.6a (1.5-4.3)</td>
<td>2.5a (2.1-4.6)</td>
<td>600a (200-850)</td>
</tr>
</tbody>
</table>

serum Cu concentrations were typified by the Hundreds of Short, Joyce, Coles, Spence and Wooloombool (Table 2). These Hundreds have predominantly acidic sandy soils ranging from shallow sand over clay to deep sandy rises. Hannam et al. (1982) found that pasture and sheep production on similar soils was maintained by Cu fertiliser applied up to 23 years earlier. The serum Cu concentrations from the Hundreds of Senior Archibald/Coombe and Colebatch (Fig. 1), where Hannam et al. (1982) conducted their work, showed low risk with 37% of herds with serum Cu of 7 pmol/L or less. Despite the wide and repeated usage of Cu-enriched fertilisers, this study has shown that there are herds at risk to Cu deficiency. On the peat soils of the region, Mo-induced Cu deficiency reduces livestock production (Deland et al. 1986). However, it has not been clear what factors (low Cu concentrations in the diet or antagonists affecting Cu absorption) cause Cu deficiency in livestock grazing on the other soil types.

Copper concentration in pasture

The Cu concentrations in pastures sampled from the red gum soil type were significantly higher than those from all soil types except the ground water rendzina (Table 3). This supports the suggestion by Tiver (1955) that these were 2 soil types in the South East that did not need applications of Cu for pasture development. Individual results from subterranean and strawberry clover samples (Table 3) indicate that plants from only 13 samplings out of 213 could be considered Cu deficient [Cu concentration <4.0 mg/kg DM (McFarlane 1989)]. These 13 samplings were from 5 paddocks of sand over clay and 3 paddocks of calcareous sand. Samples, at other times, from 4 of these paddocks had concentrations >4.0 mg Cu/kg DM.

There was no clear relationship between the pasture Cu concentration and the total Cu fertiliser applied, or the time since Cu fertiliser was last applied on an individual paddock. This was because the Cu concentrations in clover growing in individual paddocks varied within seasons and between years, as illustrated by repeated samplings taken at Kybybolite Research Centre (Table 4). Kybybolite Research Centre is situated on red gum soil (predominantly solodised solonetz) which naturally (paddock Pt. 2, Table 4) supplies adequate Cu for scattered subterranean clover plants (McFarlane 1989) and sheep production (Langlands et al. 1989).

Table 4. The variation in mean mineral concentrations of subterranean clover whole tops from five paddocks on red gum soil type at Kybybolite Research Centre over three years

Mean values each comprised of 5-10 samples and the range (in parentheses) of mean concentrations of samples each comprised of 5-10 samples

<table>
<thead>
<tr>
<th>Paddock</th>
<th>Fertiliser history</th>
<th>No. of samplings (samples)</th>
<th>Cu (mg/kg)</th>
<th>Mo (mg/kg)</th>
<th>S (g/kg)</th>
<th>Fe (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt. 1</td>
<td>90 kg P/ha.year, 0.5 kg Cu/ha 1959</td>
<td>12 (71)</td>
<td>7.5 (5.8-9.2)</td>
<td>1.0 (0.2-3.1)</td>
<td>3.2 (2.6-4.3)</td>
<td>400 (200-850)</td>
</tr>
<tr>
<td>Pt. 2</td>
<td>No fertiliser applied</td>
<td>13 (95)</td>
<td>11.7 (9.2-14.5)</td>
<td>4.1 (0.9-6.8)</td>
<td>2.4 (2.0-2.8)</td>
<td>660 (70-1500)</td>
</tr>
<tr>
<td>N5</td>
<td>90 kg P/ha.year, 1.0 kg Cu/ha 1959</td>
<td>12 (90)</td>
<td>10.7 (7.9-12.9)</td>
<td>1.7 (0.4-4.5)</td>
<td>2.3 (1.7-3.1)</td>
<td>560 (260-1630)</td>
</tr>
<tr>
<td>S6</td>
<td>90 kg P/ha.year</td>
<td>13 (105)</td>
<td>11.8 (8.8-15.9)</td>
<td>0.9 (0.5-1.3)</td>
<td>3.5 (3.0-3.9)</td>
<td>280 (220-330)</td>
</tr>
<tr>
<td>S7</td>
<td>90 kg P/ha.year, 1.0 kg Cu/ha 1966</td>
<td>11 (83)</td>
<td>9.9 (5.2-12.8)</td>
<td>2.3 (0.8-5.2)</td>
<td>2.5 (2.1-3.3)</td>
<td>690 (160-2300)</td>
</tr>
</tbody>
</table>

A Phosphorus applied as superphosphate.
been applied (paddock S6, Table 4) and improved the dilution of Cu concentration in pasture due to pasture replacing native species, there is no evidence of Cu concentrations in Table 4), there is no increase in already adequate Cu concentrations in pasture. In some seasons, a proportion of lambs show clinical signs of Cu deficiency at Kybybolite Research Centre. Where Cu has been applied (paddocks Pt. 1, N5, and S7, Table 4), there is no increase in pasture production (Hannam and Reuter 1977).

Clover samples were collected from 6 paddocks (14 samplings totalling 107 samples) in the Hundred of Short, which had a low incidence of low serum Cu concentrations (Table 2). The mean Cu concentration of subterranean clover was 6.5 ± 2.6 mg/kg DM, ranging from 3.0 to 13.9 mg/kg. This suggests that, at times, the pasture Cu concentration would be marginal for maintaining Cu adequacy in livestock (Langlands et al. 1981; Grace 1983), especially when it is considered that grasses generally have lower Cu concentrations than clovers (Kubota 1983). It would indicate an uncomplicated system of Cu absorption without the presence of dietary antagonists.

Molybdenum concentration in pasture

The Mo concentrations of pasture ranged from 1.6 to 21.8 mg/kg DM on calcareous sand, 4.7 to 16.2 mg/kg DM on peat and from 0.1 to 5.4 mg/kg DM on other mineral soils (Table 3). Suttle (1981) found that Mo concentrations as low as 2 mg/kg in the diet had a measurable negative effect on the Cu status of sheep in the presence of adequate S. Pasture alone will rarely meet the Cu requirements of growing or adult cattle when it contains >2 mg Mo/kg DM (Suttle 1983). Low serum Cu concentrations from the survey were associated with pasture Mo concentrations of this order.

While high Mo concentrations had been associated with Cu deficiency in cattle on peat soils (Hannam and Marrett 1976; Deland et al. 1986) this association has not been recognised on other soil types in the region. With the exception of the calcareous sands and ground water rendzinas, which are alkaline, it was thought that the other soils were generally Mo responsive due to an acidic reaction in their upper profiles, and their sandy texture. Molybdenum is readily leached from sands (Jones and Belling 1967) while its availability to plants increases in alkaline soils (Caldwell 1971). The Fe content of some acidic soils (especially the red gum soils) in the region may immobilise Mo, resulting in increased retention and availability of Mo (Jones and Belling 1967). These acidic soils are often shallow and are prone to waterlogging in winter, further increasing the Mo in soil solution and in plants (Kubota et al. 1963).

Mean Mo concentrations of clover (1.3 mg/kg, with concentrations ranging from 0.2–6.9 mg/kg) collected during an acid soil survey (Hodge and Lewis 1989) (pH<5.5, 1:5, soil:water) were similar to those on other soil types except the peat and calcareous sand (Table 3). The highest Mo concentration determined was 6.9 mg/kg in subterranean clover on a soil of pH 5.1.

Bingley and Anderson (1972) found the Cu/Mo concentration ratio of the improved pasture in their study was below 2.0. This was not the case in the present survey, except on the peat and calcareous sand. The Mo concentrations in sampled paddocks at the Kybybolite Research Centre differed by as much as a factor of 15 (Pt. 1, Table 4) and by at least a factor of 3 (S6, Table 4), and differences were only attributable to different environmental conditions at the times of sampling. Under cold and wet conditions Mo is more readily available to plants than Cu (Barrow and Shaw 1975; Kubota 1983), and in wet years Cu deficiency increases in sheep (Hannam and Reuter 1977).

Sulfur concentration in pasture

Total S concentrations in pasture on peat (3.6 g/kg DM) were significantly higher than those in pasture from the other soil types (Table 3). Pasture S concentrations over all soil types were between 1.5 and 5.0 g/kg DM, consistent with concentrations associated with a limiting effect on Cu absorption independent of Mo (Gawthorne 1987).

Mean S concentrations in clover (1.8 g/kg with concentrations ranging from 1.0–3.2 g/kg) were significantly lower in the soil acidity survey (Hodge and Lewis 1989) than those measured in the Cu status survey (Table 3). Differences may be due to a seasonal variation. Samples for the Cu status survey were collected from July to September, whilst the sampling in the soil acidity survey was undertaken between October and December. Sulfur concentration in clover (Clarke and Lewis 1974) and inorganic sulfate in pasture (Bingley and Anderson 1972) decrease in spring. The potential for sulfide generation is also reduced in dry summer pasture due to the decreased solubility of proteins (Gawthorne 1987).

Iron concentration in pasture

Pastures sampled from red gum and ground water rendzina soils had the highest Fe concentrations, in excess of 500 mg/kg DM (Table 3) and ranging up to 2300 and 1000 mg/kg on the red gum soil and rendzina, respectively. These concentrations could reduce the absorption of Cu in grazing ruminants (Phillippo et al. 1987). High concentrations of Fe most likely reflect soil contamination of the samples from both soils. Iron deficiency can occur in plants growing on the alkaline rendzina soils in cold and wet conditions. These rendzina soils have low concentrations of Fe available to plants, while having high concentrations of total Fe. In contrast to Mo, Fe reduces liver and plasma Cu concentrations.
but does not appear to affect clinical appearance, growth or reproductive performance in cattle (Campbell et al. 1974; Humphries et al. 1983; Suttle et al. 1984; Phillippo et al. 1987).

Soil ingestion by livestock

High Fe concentrations in pasture analysis indicated soil contamination (Table 3). Soil ingestion and its role in Cu metabolism is not well understood but it occurs at times of low pasture availability in autumn and winter (Suttle et al. 1975; Mayland et al. 1977; Jarvis and Austin 1983; Suttle et al. 1984). Soil intake may also occur when stock are eating grain in stubble paddocks or when sheep are eating subterranean clover burr. Ingestion of soil may reduce Cu availability, either through an association of dietary Cu with one or more of the soil colloids (by sorption or complex formation), or through an interaction with S and Fe or other soil elements such as Mo, Zn or Cd.

Conclusions

The Mo and S concentrations found in the majority of pastures sampled in this study were not as high as those associated with the depletion of Cu reserves in livestock (Bingley and Anderson 1972; Langlands et al. 1981). The low serum Cu concentrations we have observed indicate a significant number of herds at risk to Cu deficiency and this appears to be associated with moderate Mo, S and Fe concentrations in pasture, on the mineral soils, rather than low pasture Cu concentrations. On the peat and calcareous sand the Mo and S concentrations in pasture were high enough to deplete the Cu reserves of grazing animals (Langlands et al. 1981; Bingley and Anderson 1972).

Where Cu concentrations in pasture approach 10 mg/kg and Cu deficiency still occurs in livestock, application of Cu enriched fertiliser to pasture is not appropriate as uptake in pasture plants is inefficient above this concentration (McFarlane 1989). In such cases, Cu deficiency should be treated by the administration of Cu directly to the animals.

On soils with pH <5.5, significant responses in pasture growth to lime applications have been observed (Lewis and Hodge 1984). The greater use of lime on pasture is appropriate. The survey proved invaluable in determining the geographical distribution of Cu status in the region.

Acknowledgments

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