# Effect of the application of copper to pasture on the copper status of grazing weaner, yearling and mature hinds

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#### Summary

- Copper (Cu) is the most important trace element for deer. Clinical signs of Cu deficiency include enzootic ataxia (a nerve disorder) and osteochondrosis (a bone disorder).
- The application of 6 kg copper sulphate/ha (1.5 kg Cu/ha) applied in March increased pasture Cu concentrations 20-25 mg Cu/kg DM at 4 weeks after treatment before decreasing to a baseline concentration of 7 mg Cu/kg DM after 70-80 days.
- The application of 12 kg copper sulphate/ha (3.0 kg Cu/ha) applied in March increased pasture Cu concentrations 45-60 mg Cu/kg DM at 4 weeks after treatment before decreasing to a baseline concentration of 7 mg Cu/kg DM after 130-150 days.
- The deer were grazed on the Cu treated pastures 4 weeks after the Cu application to allow the Cu to be washed into the soil by the rain and to allow plant Cu uptake by the pasture regrowth.
- The 6 kg copper sulphate/ha treatment had no significant effect when compared with untreated controls on the Cu status, as determined by changes in serum and liver Cu concentrations, of the deer.
- The 12 kg copper sulphate/ha treatment had a significant effect when compared with untreated controls on the Cu status, as determined by changes in serum and liver Cu concentrations, of the weaner, yearling and mature hinds.
- The untreated controls were at risk of being Cu deficient as their serum and liver Cu concentrations were <5 µmol/L and <80 µmol/kg fresh tissue during the winter early spring period.

- Increasing the Cu status of pregnant hinds, by Cu topdressing, also markedly increased the Cu status of their fawns from birth to weaning. Young deer are most prone to Cu deficiency.
- The uptake of Cu by pasture can be variable as factors such as application rate, soil type and botanical composition can influence pasture Cu concentrations.
- An autumn application of Cu at the rate of 12 kg copper sulphate/ha is very effective in maintaining an adequate Cu status of grazing deer for up at least 10 months provided that the pasture Cu concentrations reach at least 45 mg Cu/kg DM and remain at this level for at least 60-100 days.

#### Introduction

Copper (Cu) deficiency is the most important trace element problem in deer (Grace and Wilson 2002). Signs of the deficiency include enzootic ataxia and osteochrondrosis which then impact on animal performance. The Cu status of deer can be assessed from tissue Cu reference ranges, namely for serum Cu (µmol/L) the criteria are <5 deficient, 5-8 marginal and >8 adequate, and likewise for liver (µmol/kg fresh tissue) the criteria are <60 deficient, 60-100 marginal and >100 adequate (Wilson and Grace 2001). These tissue Cu reference ranges have been based on the presence and absence of clinical signs of Cu deficiency.

The Cu status of deer shows a seasonal pattern, with serum and liver Cu concentrations being the lowest in the late winter/early spring and highest in summer.

Forages such as chicory with a Cu content of at least 11 mg/kg DM meet the dietary Cu requirements for deer, where as ryegrass/white clover pastures containing 5-8 mg/kg DM may not provide an adequate Cu intake for deer.

To prevent against Cu deficiency many deer farmers supplement with Cu. Topdressing pastures with Cu has been shown to increase and maintain the Cu status of sheep and cattle for varying periods. There are no data for deer.

#### Objective

To evaluate the use and efficacy of Cu topdressing to increase and maintain the Cu status of weaner, yearling and mature hinds as an approach to prevent Cu deficiency in grazing deer.

#### **Materials and Methods**

#### Animals

The study was run on the AgResearch deer unit, Lockwood Road, near Palmerston North. In Year 1 (2000) weaner hinds were placed on the trial in April. In Year 2 (2001) they were mated in April/May and returned to the trial, as yearling hinds, in April. Also in Year 2 a further group of mature pregnant hinds were placed on the trial in April.

#### Treatments and experimental design

In Year 1 (2000) there were 3 treatments:

- Group 1 Control, no Cu application
- Group 2 6 kg copper sulphate/ha (1.5 kg Cu/ha)
- Group 3 12 kg copper sulphate/ha (3.0 kg Cu/ha)

The copper sulphate was mixed with 15% potash superphosphate and the Cu amended fertiliser applied at a rate of 250 kg/ha on 1.1 hectare paddocks in mid March 2000. There were 2 paddocks per treatment to allow for rotation grazing. Four weeks after the Cu application in Year 1 eleven weaner hinds per treatment were randomised on to the untreated and Cu-treated pastures and remained on the trial for 11 months.

In Year 2 (2001) there were 2 treatments and 2 age groups of deer:

- Group 1 Control, no Cu application
- Group 2 12 kg copper sulphate/ha (3.0 kg Cu/ha)

The Cu was reapplied using the method described as for Year I, and on to the same treatment paddocks in mid March 2001. The eleven weaner hinds from Year 1, now yearling hinds, were mated during April/May and placed on to their respective treatments in mid April. In addition, groups of 10 pregnant mature hinds (mated in early April and diagnosed as pregnant) were also placed on the 2 treatments in mid

April. The yearling hinds calved in December 2001 and the mature hinds calved in November 2001. All fawns were weaned in mid March 2002.

The mature pregnant hinds were added to the study because the pregnancy rate of the yearling hinds could have been low and it was important that there were an adequate number of pregnant hinds per treatment group for the study.

#### **Collection of samples**

The pasture samples were collected just before treatment and at monthly intervals after the application of the Cu amended fertiliser.

Blood samples and liver samples using a liver biopsy technique were collected just before treatment and at monthly intervals in the weaner hinds (Year 1), and in the yearling and mature hinds (Year 2) during mid April, July, September, December, January and March.

All samples were analysed for Cu.

#### Statistical methods

Significant differences between treatments were determined by repeated measures of analysis of variance.

#### Results

#### Pasture Cu concentrations

The changes in pasture Cu concentrations after the application of Cu in Year 1 and in Year 2 are shown in Figures 1 and 2.

In Year 1 (2000) at Day 28 after the Cu application, the pasture Cu concentrations were 35 and 25 mg/kg DM for the 12 and 6 kg copper sulphate/ha treatments, respectively, before they decreased to baseline concentrations. At Day 80 all pasture Cu concentrations were similar at 6-8 mg Cu/kg DM.



Figure 1. Effect of Cu topdressing in mid March (2000) on pasture Cu concentrations

Time after application (days)





In Year 2 (2001) at Day 28 the 12 kg copper sulphate/ha treatment increased the pasture Cu concentration to 60 mg Cu/kg DM and maintained pasture Cu concentration above 25 mg/kg DM for 110 days, while at Day 160 there was no difference between pastures in Cu concentrations of 7-9 mg Cu/kg DM.

#### Tissue Cu concentrations of deer

The effect of Cu topdressing on the serum and liver Cu concentrations of weanling hinds (Year 1, 2000) is illustrated in Figures 3 and 4.



Figure 3. Effect of Cu topdressing in March on the serum Cu concentrations of weaner deer

A seasonal decrease in the serum Cu concentrations (ie <5.5  $\mu$ mol/L) was observed in the weaner hinds during late winter/early spring after which they increased in the summer to 10  $\mu$ mol/L. A Cu application of 6 kg copper sulphate/ha had no significant effect on serum Cu concentrations of weaner hinds when compared with untreated control animals. (Note vertical bars are standard errors of the mean). However, a Cu application of 12 kg copper sulphate/ha significantly increased the serum Cu concentrations of the deer at Days 157 and 185 (mid August and mid September) and ensured that their Cu status was adequate, that is, >8.0  $\mu$ mol/L for the duration of the trial.



# Figure 4. Effect of Cu topdressing in March on the liver Cu concentrations of weaner deer

A seasonal decrease in the liver Cu concentrations (ie >80  $\mu$ mol/kg fresh tissue) was observed in the weaner hinds during late winter/early spring, after which they then increased slightly in the summer. A Cu application of 6 kg copper sulphate/ha had no significant effect on liver Cu concentrations of weaner hinds when compared with untreated control animals. (Note vertical bars are standard errors of the mean). However, a Cu application of 12 kg copper sulphate/ha significantly increased the liver Cu concentrations of the deer at Days 157, 185, 214 and 250 (mid August to mid November) and ensured that their Cu status was adequate, that is, liver Cu >100  $\mu$ mol/kg fresh tissue for the duration of the trial.

The effect of Cu topdressing on the serum and liver Cu concentrations of pregnant yearling and mature hinds (Year 2) is illustrated in Figures 5, 6, 7 and 8.





The serum Cu concentrations of the yearling hinds decreased during mid July and August to <4  $\mu$ mol/L and these animals were at risk of becoming Cu-deficient. Grazing pastures topdressed with Cu at a rate of 12 kg copper sulphate/ha (3 kg Cu/ha) maintained serum Cu concentrations >10  $\mu$ mol/L and these deer had an adequate Cu status. The Cu status of the hinds also had significant impact on the Cu status of their fawns. The higher serum Cu concentrations of hinds on the Cu topdressed pasture were reflected in the higher serum Cu concentrations seen in their fawns.



Figure 6. Effect of Cu topdressing in March on the serum Cu concentrations of mature hinds and their fawns

The Cu status of the control mature hinds, based on serum Cu concentrations, was higher than that of the yearling hinds because they had been supplemented with Cu the previous year, where as the yearlings had not, as they were the untreated controls in Year 1 of this study (see Figure 3). Nevertheless, the mature deer on the Cu-topdressed pasture had significantly higher serum Cu concentrations at Day 183 (mid July). As the Cu status of the control mature hinds was marginal to adequate no Cu treatment effect was seen on the Cu status of their fawns.



## Figure 7. Effect of Cu topdressing in March on liver Cu concentrations of yearling hinds and their fawns

The liver Cu concentrations of the yearling hinds decreased during mid July and August to <60  $\mu$ mol/L, and these animals were at risk of becoming Cu-deficient. Grazing pastures topdressed with Cu at a rate of 12 kg copper sulphate/ha (3 kg Cu/ha) maintained liver Cu concentrations at >200  $\mu$ mol/L and these hinds had an adequate Cu status.

Regardless of Cu treatment, the Cu concentrations of the 3-week old fawns were greater than those of their dams. The Cu status of the hinds had significant impact on the Cu status of their fawns, as the higher liver Cu concentrations of hinds on the Cu topdressed pasture were reflected in the higher liver Cu concentrations of their fawns.

Further the increase in the liver Cu stores of the fawns from the high Cu hinds was large, and therefore this ensured a more than adequate Cu status of their fawns from birth to weaning.





The Cu status of the control mature hinds was high because they had been treated with Cu the previous year. However, their liver Cu concentrations decreased markedly from 700 to 80  $\mu$ mol/kg fresh tissue during April to September and remained in the marginal Cu status range for the rest of the trial. In contrast, the liver Cu concentrations of the mature hinds on the Cu topdressed pasture remained at >200  $\mu$ mol/kg fresh tissue and were well within an adequate Cu status range for the 350-day study. There was no effect of hind Cu status on the fawn Cu status but the 3-week old fawn liver Cu concentrations were much higher than that their dams regardless of Cu treatments.

#### Discussion

Growth responses in deer to Cu supplementation are not very common, while the clinical signs of Cu deficiency such as enzootic ataxia (a nerve disorder) and osteochondrosis (a bone disorder) have been documented and related to serum and liver Cu concentrations. On the basis of these observations the following tissue Cu references have been established. At serum Cu concentrations of <5, 5-8 and >8  $\mu$ mol/L the Cu status of deer are classified as deficient, marginal and adequate, respectively. Likewise, for liver Cu concentrations the respective values are <60, 60-100 and >100  $\mu$ mol/kg fresh tissue (Wilson and Grace 2001).

This relationship between Cu intake or Cu status and serum and liver Cu concentrations means that the efficacy of a Cu supplementation approach such as applying Cu to pastures can be evaluated from changes in tissue Cu concentrations. The application of 12 kg of copper sulphate/ha in the autumn was very effective in increasing and maintaining the Cu status, as determined by changes in serum and liver Cu concentrations, of weaner, yearling and mature hinds for about a year. An application rate of 6 kg copper sulphate/ha, the present fertiliser industry recommended rate, resulted in no significant changes in the Cu status of the grazing deer.

The herd used in this study were ideal to evaluate the effect of Cu topdressing on the Cu status of deer as the untreated animals, particularly the yearling hinds, had a low Cu status (ie serum Cu <4  $\mu$ mol/L and liver Cu <60 mol/kg fresh tissue) and were at risk of being Cu deficient. In previous years osteochondrosis had been diagnosed in young deer in this herd.

Many factors such as application rate, botanical composition and soil type can influence the pasture uptake of Cu. For Cu topdressing to be effective, at 28 days after application, the pasture Cu concentrations must be at least 45 mg Cu/kg DM and remain elevated for 60 to 100 days. After the application of Cu, 28 days were allowed for the rain to wash the Cu into the soil and then to be taken up by the pasture regrowth before the treated pastures were grazed with the deer.

There could be a concern the grazing of pastures containing 45-60 mg Cu/kg DM with sheep could cause deaths, because sheep are more sensitive to Cu toxicity than cattle or deer. Therefore, it is not recommended that sheep should graze high Cu pastures. However, if this does occur as a result of human error the situation may not be so serious for pasture-fed sheep. A recent study (Grace et al 1998) with young Romney sheep grazing pastures containing up to 140 mg Cu/kg DM for 99 days showed that while liver Cu concentrations reached 7000  $\mu$ mol/kg fresh tissue, no deaths occurred and their mean liveweight gain was 95 g/day. Liver Cu concentrations of >7000  $\mu$ mol/kg fresh tissue are indicative of Cu toxicity and had the above lambs been exposed to stress, bad weather or a feed shortage, it is most likely that deaths would have occurred. However, as the Romney lambs grazing pastures containing 45-60 mg Cu/kg DM for 99 days had liver concentrations of 5000  $\mu$ mol/kg fresh tissue, there is a small safety margin for Romney sheep grazing pastures that have been topdressed with 12 kg copper sulphate/ha.

However for some breeds such as the Texel, which are more efficient at absorbing Cu, grazing Cu topdressed pastures might be a problem. The situation needs to be studied.

Copper topdressing (12 kg copper sulphate/ha) is an easy, cost effective way to prevent Cu deficiency in deer where a comprehensive fertiliser programme is in place on the farm. The application of Cu amended fertilisers in conjunction with autumn save pasture means that a large enough area of high Cu pasture can be made available to the herd in the autumn. The higher Cu intakes at this time are effective to counter the late winter/early spring decline in the Cu status of deer as well as ensuring good liver Cu stores in the developing foetus. Fawns born with high liver Cu concentrations will have an adequate Cu status from birth to weaning. The above scenario was readily achieved by Cu topdressing.

The cost of topdressing with 12 kg copper sulphate/ha is about \$25.00/ha, which is very similar to that of Cu0 needles (based on 10 hinds/ha with cost of a 10 g Cu0 dose at \$2.50). Further, the extra cost and time needed to handle the deer for dosing CuO needles makes Cu topdressing even more attractive.

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#### References

- Grace, N.D.; Knowles, S.O.; Rounce, J.R.; West, D.M.; 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.
- **Grace, N.D.; Wilson, P.R.** 2002: Trace element metabolism, dietary requirements, diagnosis and prevention of deficiencies in deer. *New Zealand Veterinary Journal* (submitted).
- Wilson, P.R.; Grace, N.D. 2001: A review of tissue reference values used to assess the trace element status of farmed red deer (*Cervus elaphus*). *New Zealand Veterinary Journal* 49: 126-132.