Effect of increasing Mo intakes on the Cu status of deer and the impact of Cu topdressing on the Cu status of animals grazing high Mo pastures

Report

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Summary

The metabolism of Cu is complex, and it has been well documented that increasing intakes of Mo, in the presence of S, decrease liver Cu stores and increase the dietary Cu requirements of cattle and sheep. The consequences of the Cu x Mo interaction in grazing deer have not been studied. The objectives of this study were to (1) study the effect of increasing Mo intakes on the Cu status of deer; and (2) evaluate the effect that an application of a Cu-amended fertiliser has on the Cu status of deer grazing high Mo pastures.

The experimental design was a 3 x 2 factorial with three treatments of Mo and two treatments of Cu. In mid-April 2003, sodium molybdate was applied at the rates of none, 0.5 and 1.0 kg/ha to give pastures with Mo concentrations of 1.5-2 (control), 3-5 (low), and 10-13 (high) mg/kg DM. In late May, 12 kg copper sulphate/ha was applied over half the area of the experimental Mo pastures, so that within a Mo treatment there was an untreated control and a Cu treatment. From 60 weaner hinds, 6 groups of 10 animals were randomly selected and placed on each of the 6 treatments in late June. Changes in the Cu status of the deer were assessed from changes in serum and liver Cu concentrations prior to the animals being placed on the experimental pastures and at about monthly intervals.

At about 60 days after the application of Mo-amended fertiliser, pasture Mo concentrations were increased from a baseline level of 1.5-2, to 3-5 and 10-13 mg Mo/kg DM for the low and high Mo treatments, respectively, and were maintained at these concentrations for at least 180 days. Pasture Cu concentrations were increased from a baseline level of 6-9 to 95 mg Cu/kg DM at 30 days after the application of the Cu-amended fertiliser, after which they decreased but remained above 40 mg Cu/kg DM for the next 50 days, before decreasing to baseline concentrations at 125 days after treatment.

As the Cu topdressing in this study had no overall significant effect on the Cu status of the deer, the serum and liver Cu data from deer grazing untreated and Cu-treated pastures were pooled within a Mo treatment. When compared with deer on the control Mo pasture (1.5-2 mg Mo/kg DM), the serum and liver Cu concentrations of animals grazing the 3-5 and 10-13 mg Mo/kg DM pastures decreased markedly within 30 days, after which the decline in tissue Cu concentrations was slower over the next 75 days, when the trial was terminated. For example, the serum Cu concentrations of the control deer decreased from 9.0 to 7.5 µmol/L over the 105-day trial, while in deer on the low and high Mo-treated pastures the serum Cu decreased from an initial 9 µmol/L to 4 and 3 µmol/L at Days 30 and 105, respectively, after treatment. Likewise, with liver Cu concentrations a decrease occurred in all treatments over

the trial. In the control deer the liver Cu values were 131, 120 and 52 µmol/kg fresh tissue, while in the animals on the low and high Mo-treated pastures the liver Cu values were 131, 59 and 20 µmol/kg DM prior to treatment, and 30 and 105 days after treatment, respectively. Deer with serum and liver Cu concentrations of <5 µmol/L and <60 µmol/kg fresh tissue, respectively, are Cu-deficient.

It was concluded from this study that increasing the Mo intakes of deer, that is grazing pastures containing >3 mg Mo/kg DM, resulted in a marked decrease in their Cu status and therefore the impact of the Cu x Mo interaction is very important in the Cu nutrition of deer. Copper topdressing was observed in this trial not to be effective in countering the increased Mo intakes.

Introduction

The metabolism of Cu is complex because dietary factors such as increasing Mo intakes, in the presence of S, can decrease the absorption and hepatic storage of Cu in cattle and sheep. There a dearth of data on the impact of the Cu x Mo interaction in deer, even though Cu is one the most important trace elements in deer nutrition. In cattle, when pasture Mo concentrations are >2 mg Mo/kg DM there is a marked effect on the Cu absorption, and liver Cu concentrations are very low (Lee 2002). It is not uncommon for pastures, depending on soil type, to have Mo concentrations >3 mg Mo/kg DM, and therefore Mo-induced Cu deficiency could be important on some deer farms.

Copper supplementation strategies involving Cu-amended fertilisers and orally dosed CuO particles have been shown to be effective in maintaining an adequate Cu status of grazing deer when pasture Mo are low (Grace et al 2002).

Objectives

1. Study the effect of increasing Mo intakes, that is, pastures containing high concentrations of Mo, on the Cu status of deer.
2. Evaluate the effect of an application of a Cu-amended fertiliser on the Cu status of deer grazing high Mo pastures.

Materials and Methods

Experimental treatments

The study was carried out on the AgResearch Aorangi Deer Unit near Palmerston North. The design of the trial was a 3 x 2 factorial with three levels of Mo and two levels of Cu. Six 1.1 ha paddocks with a ryegrass/white clover pasture were used in the study. On 18th April 2003, sodium molybdate was applied at the rate of none, 0.5 and 1.0 kg/ha on 2 paddocks per Mo treatment previously selected at random. On 26th May, copper sulphate was applied at the rate of none or 12 kg/ha, such that within each Mo treatment there was an untreated and a Cu-treated pasture.

To ensure a satisfactory distribution of Cu over the pasture the Cu-amended fertiliser was prepared as follows. The copper sulphate, which was sourced from Ravensdown Feilding, appeared to be too coarse to ensure a satisfactory mix with 15% potassiac superphosphate, and therefore it was further pulverized to a powder before 2.4 kg copper sulphate were mixed with 50 kg fertiliser in a concrete mixer for about 10 minutes. The Cu-amended potassiac superphosphate was applied at a rate of 262 kg/ha to provide 12 kg copper sulphate /ha.

At the end of June, 60 weaner hinds were randomly divided into 6 groups of 10 animals and a group was placed on each of the 6 treatments. In summary the treatments were:

Group 1 (Control)	No Mo and no Cu applied
Group 2	No Mo and Cu applied
Group 3	Low Mo and no Cu applied
Group 4	Low Mo and Cu applied
Group 5	High Mo and no Cu applied
Group 6	High Mo and Cu applied

The deer grazed the experimental pastures for 105 days, while pasture Mo and Cu concentrations were monitored for 240 days. All procedures involving the experimental use of animals were approved by the Crown Research Animal Ethics Committee (Palmerston North).

Collection of samples

Blood and liver biopsy samples were taken from the deer just prior to being placed on the treatments and at about monthly intervals. Blood samples were taken from the jugular vein using a 10 ml vacutainer containing no anticoagulant. The blood samples were centrifuged at 2000 g for 20 minutes, the serum collected and stored at 4°C for Cu determinations. The 60-100 mg liver biopsy samples were taken using the procedures described by Dick (1944), blotted dry, and stored at -5°C for Cu determinations.

The pasture samples were collected at about 2-weekly intervals by plucking small samples about every 10 m along a 150 m transect. The bulk sample was then thoroughly mixed, subsampled, and stored at -5°C for Mo and Cu determinations.

Chemical analysis

The Cu and Mo were determined by inductively coupled plasma atomic emission spectrometry (ICP/AEC) as described by Lee (1983).

Statistical analysis

The difference between treatments was determined by analysis of variance using the statistical procedures of SAS 8.02.

Results

The particle size distribution of the copper sulphate used in the preparation of the Cu-amended fertiliser applied to the trial pastures of this study is shown in Table 1.

Table 1. Particle size distribution (%) of copper sulphate used in the Cu-amended fertiliser.

Seive size (mm)	>2.0	>1.0	>0.5	>0.25	>0.15
Particle size	15.9	60.6	23.0	0.3	0.2

The effect of applying none, 0.5 and 1.0 kg sodium molybdate to autumn pasture on herbage Mo concentrations is illustrated in Figure 1.

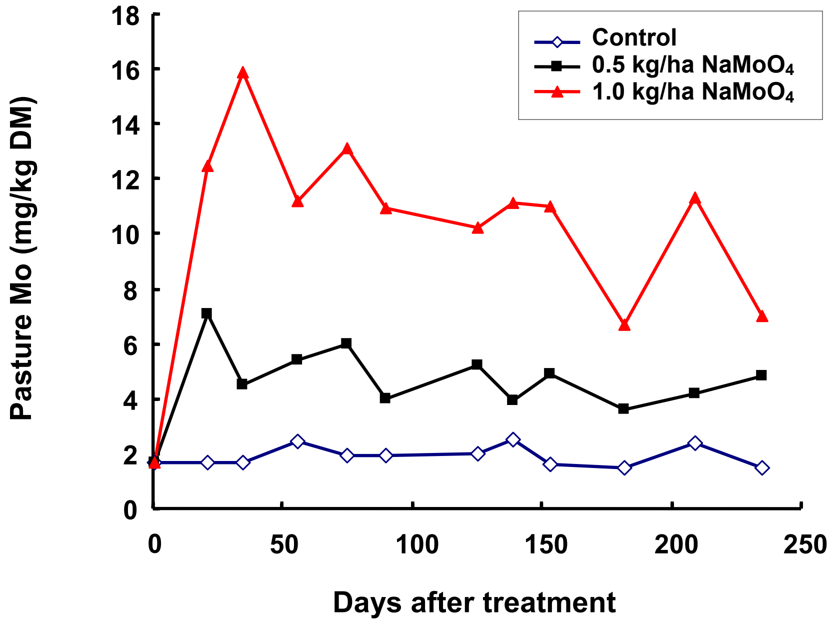

Figure 1. Effect of Mo-amended fertiliser on pasture Mo concentrations.

The control baseline Mo concentrations were 1.5-2 mg Mo/kg DM, and from Day 60 the range of Mo concentrations in pasture treated with 0.5 and 1.0 kg sodium molybdate/ha were 3-5 and 10-13 mg Mo/kg DM, respectively, for up to 150 days.

The effect of applying none or 12 kg copper sulphate/ha on pasture Cu concentrations is shown in Figure 2.

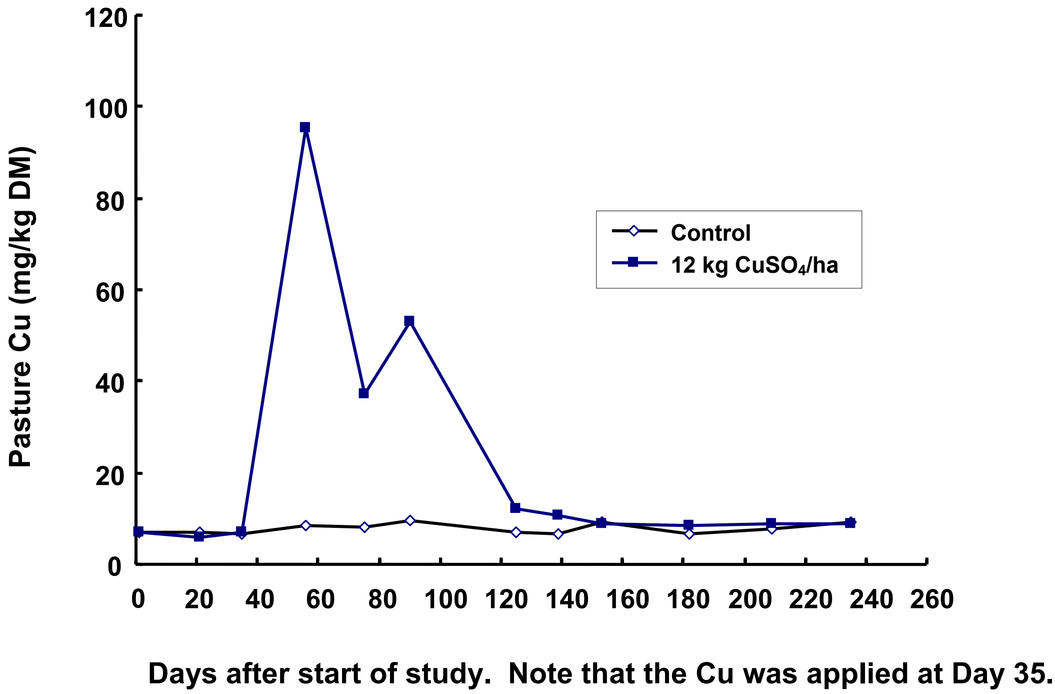

Figure 2. Effect of Cu-amended fertiliser on pasture Cu concentrations.

The application of Cu increased the pasture Cu concentrations to 95 mg/kg DM at 30 days, and maintained levels above 40 mg/kg DM for a further 50 days. The deer would, therefore, have markedly increased Cu intakes for at least 80 days.

As the Cu treatment, that is, the Cu-amended fertiliser, had no significant effect on the deer serum or liver Cu concentrations, the Cu tissue data from deer grazing untreated and Cu-treated pastures were pooled within a Mo treatment.

The effect of increasing Mo intakes on deer serum Cu concentrations is shown in Figure 3. The mean initial serum Cu concentration of all deer was 9.0 µmol/L. In deer on the untreated Mo pasture this increased to 9.8 µmol/L at Day 38, before decreasing to 7.8 µmol/L at Day 102. In the animals on the Mo-treated pastures the serum Cu significantly ($P<0.003$) decreased to 4.1 µmol/L at Day 38, before decreasing a little further to 3.7 µmol/L.

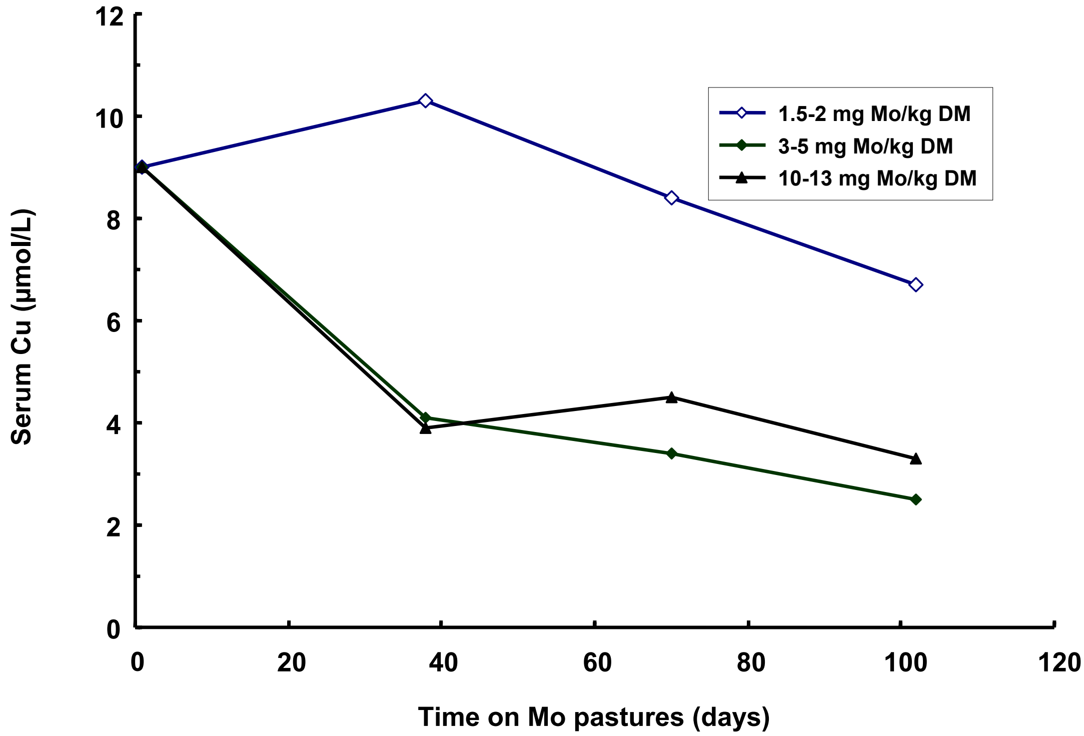

Figure 3. Effect of increasing Mo intakes on serum Cu concentrations of hinds.

The mean initial liver Cu concentration for all deer was 133 µmol/kg fresh tissue, and this decreased in all animals, regardless of the Mo treatment (Figure 4). In the deer on the untreated Mo pasture (control) the liver Cu concentrations were 120, 72 and 52 µmol/kg fresh tissue on Days 38, 70 and 102, respectively, of the trial. For the deer on the Mo-treated pastures the magnitude of the change was similar for both groups and the liver Cu concentrations were 59, 47 and 20 µmol/kg fresh tissue on Days 38, 70 and 102, respectively, of the trial.

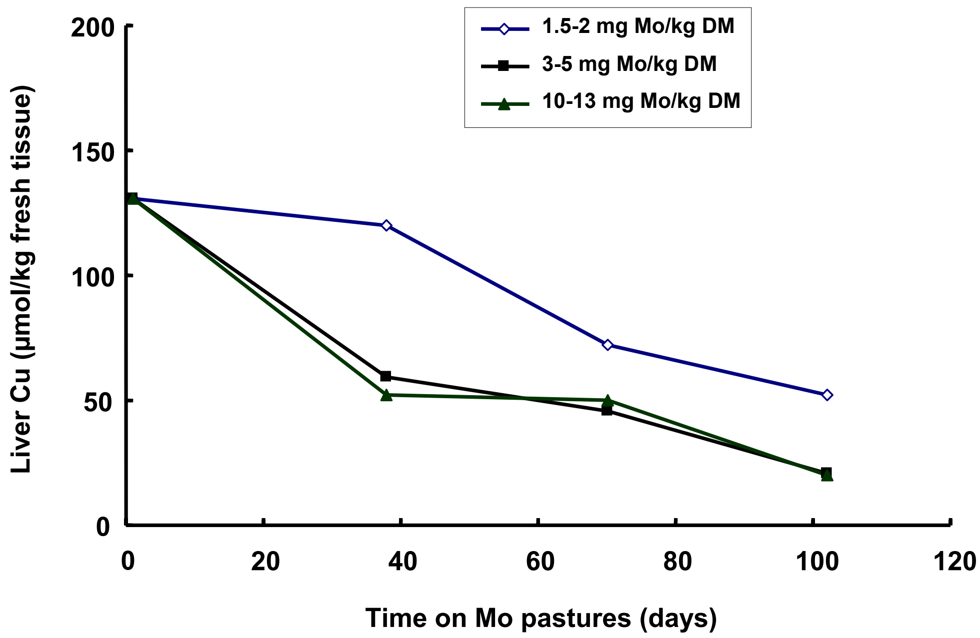

Figure 4. Effect of increasing Mo intake on liver Cu concentrations of hinds

Discussion

This study is the first to report on the impact of increasing Mo intakes on the Cu status of grazing deer, although several indoor studies had shown that Mo interfered with Cu metabolism of deer (Freudenberger et al 1987; Osman and Sykes 1989). Copper deficiency is an important problem in deer farming, and while growth responses to Cu supplementation have been reported in a few trials, the more commonly observed signs of Cu deficiency are a nerve disorder (enzootic ataxia) and/or a bone disorder (oestochondrosis) in young animals. This study confirmed that a Cu x Mo interaction occurs in deer as has been already documented in cattle and sheep (Suttle 1991). It is a very important finding, as it may assist in explaining some of the Cu nutrition problems in deer, and enable a more strategic Cu supplementation programme to be put in place.

The Cu status of deer can be assessed from changes in tissue Cu concentrations. The following reference ranges have been established (Wilson and Grace 2001). For liver, Cu concentrations of <60µmol/kg fresh tissue represent the "deficient range", wherein deer may be at risk of clinical disease. Animals in the 60-100 µmol/kg fresh tissue range are considered "marginal", while those with >100 µmol/kg fresh tissue are considered to be "adequate"; that is, they most unlikely to respond to Cu supplementation. For serum, the references are <5 µmol/L, 5-8 µmol/L and >8 µmol/L for "deficient", "marginal" and "adequate" deer Cu status.

Increasing pasture Mo concentrations from 1.5–2 mg Mo/kg DM to 3-5 mg Mo/kg DM had a marked effect, and decreased the Cu status from "adequate" to "deficient" within 38 days. The mean serum Cu concentrations decreased from 9 to 4 µmol/L (Figure 3), and mean liver Cu concentrations from 133 to 60 µmol/kg fresh tissue (Figure 4). The situation regarding the Cu status for deer grazing on the 10-13 mg Mo/kg DM pasture was found to be similar to animals on the 3-5 mg Mo/kg DM pasture. Therefore, as has been reported for cattle, pastures with 3-5 mg Mo/kg DM have a major effect on deer Cu status and any further increase in Mo intake has little additional effect on their Cu status. Pastures containing 3-5 mg Mo/kg DM are not uncommon on many deer farms in New Zealand. Pasture Mo >10 mg/kg DM have been reported in spring pasture on peat soils.

The Cu status of deer varies with the season, with serum and liver Cu concentrations being highest in the summer/autumn and lowest in early spring (Grace et al 2003), while Cu deficiency is more important in young animals. Supplementing pregnant hinds from about mid-gestation, in June, will ensure that their Cu status is maintained or increased during the late winter/early spring, while the liver Cu stores of their foetus' are also increased so that their fawns are born with a high Cu status which will continue through up to weaning in March.

In this study, no signs of Cu deficiency were observed, because deer have be Cu-deficient for many months before tissue Cu concentrations are lowered enough such that the activity of the Cu-containing enzymes are reduced and the clinical signs of Cu deficiency become apparent. However, from an animal health point of view it is essential that the Cu status of the grazing deer is maintained in the "adequate" range by the use of effective Cu supplementation programmes.

It is well documented that the application of a Mo-amended fertiliser, containing sodium molybdate, will increase pasture Mo concentrations and improve clover growth, where pasture Mo concentrations are <0.10 mg/kg DM. The recommended application rate is 50-100 g sodium molybdate/ha every 4 years. For the purpose of establishing low and high Mo experimental pastures, in this study the application rates were increased to 0.5 and 1.0 kg sodium molybdate/ha (Knowles et al 2000). The residual effects of the Mo applications are likely to persist for 4-8 years.

As has been reported in other studies (Grace et al 2003), Cu-amended fertilisers can increase and maintain high pasture Cu concentrations for varying intervals. The uptake of Cu by pasture is influenced by application rate (Morton and Smith 2000) as well as other factors such as soil type, pasture composition (Adams and Elphick 1956) and climate (i.e. soil moisture [Fleming 1973]).

In this study, the increased pasture Cu as a result of an autumn application of Cu had no effect on the Cu status of deer grazing pastures containing 3-5 or 10-13 mg Mo/kg DM. However, it was disappointing that no effect of Cu topdressing pasture with 1-2 mg Mo/kg DM on the Cu status of the deer was observed in this trial, as good results had been previously reported from two earlier studies on the same farm (Grace et al 2001; Grace et al 2003). The reasons for this lack of response in the Cu status of the deer grazing the high Cu pasture in this study are not known. The metabolism of Cu is complex as the absorption, transport and storage of Cu is associated with proteins (Vulpe and Packman 1995), while Cu is an essential co-factor for a number of cuproenzymes (Prohaska and Gybina 2004). Further, a characteristic feature of trace element studies is the unexplained variation in the magnitude of animal responses from year to year. The autumn during 2003 in the Manawatu was characterised by a severe drought which delayed the application of the Mo and Cu-amended fertilisers, and therefore the start of the trial. Further autumn pasture growth was slow.

While the use of Cu-amended fertiliser has been shown to be an effective approach to improve the Cu status of deer grazing low Mo pastures (i.e. <2 mg Mo/kg DM) (Grace et al 2003), some monitoring of changes in pasture Cu concentrations and Cu status of grazing deer is essential.

For Cu-amended fertilisers to be satisfactory and effective, some attention may have to be given to the particle size of the copper sulphate in terms of (1) mixing with the fertiliser granules to give a reasonably homogenous product; and (2) its impact on the uptake of Cu by the plant.

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