Molybdenum Toxicity in cattle: an underestimated problem.

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ABSTRACT
Molybdenum toxicity is a controversial subject. However, much of the contention is due to inappropriate diagnosis. This paper shows the flaw in using plasma copper levels alone to diagnose a molybdenum toxic condition, based on samples from >7,500 dairy cattle. Evidence that thiomolybdates are absorbed into the ruminant body and have toxic effects (molybdenum toxicity) on critical copper metallo-enzymes is detailed. The consequences are seen as impaired fertility and production. Prevention of molybdenum toxicity is achieved by reducing the iron intake of the animal and by supplementation with copper that is sacrificial in the rumen and not primarily supplied for absorption. A correct diagnosis will allow appropriate supplementation to be undertaken and the risk of toxicity from inappropriate supplementation reduced.

KEYWORDS: cattle, sheep, molybdenum toxicity, infertility, copper deficiency, iron, sulphur

INTRODUCTION
Clinical copper deficiency has historically been viewed as occurring as a primary deficiency due to a lack of copper, or as a secondary deficiency due to inhibition of normal copper homeostasis by the antagonistic minerals molybdenum, sulphur and iron (Suttle 1991). The mechanism by which these antagonists alter copper homeostasis has been the subject of much debate over the years (Suttle 2002; Suttle 2003a&b; Telfer et al 2003).
Depigmentation of hair, poor growth rates, alterations in cardiac function, anaemia, fragile bones, impaired immune function and infertility are listed as typical examples of clinical copper deficiency in cattle (McDowell, 1992). However, reports of correlations between low serum plasma copper and clinical signs have sometimes been contradictory. Rowlands et al (1977) reported that there was no correlation between blood copper and fertility. Also Mee (1991) concluded, based on blood copper levels, that hair depigmentation (brown tinge to coat) was not a clinical sign of copper deficiency. These views were also supported by Underwood and Suttle (1999). However, these views are flawed by assuming that serum/plasma copper values accurately reflect the clinical copper status of an animal. Infertility and depigmentation are clearly clinical signs (Phillippo et al 1987) and these symptoms are commonly seen in the field (Telfer et al 2003). Plasma copper concentrations have been shown to go up as well as down in response to dietary molybdenum (Suttle & Field 1968; De Plessis et al 1999; Williams, 2004).

It is undisputed that the antagonistic minerals to copper are molybdenum, sulphur and iron which react to form complexes within the rumen that can readily react with copper. Molybdenum and sulphur form a series of thiomolybdate complexes that have a high affinity for copper and iron and sulphur also complex with copper (Suttle 1991; Phillippo et al 1987).

Humphries et al (1983) and Phillippo et al (1987) clearly demonstrated hypocupraemia and reduced hepatic copper levels in cattle supplemented with molybdenum and sulphur and cattle supplemented with iron and sulphur. However, only the molybdenum and sulphur supplemented cattle had clinical signs of deficiency. Therefore, there is a difference between the mechanisms of antagonism of copper homeostasis in cattle due to molybdenum and iron.
At the time of publication Phillippo et al (1987) considered that the antagonistic effects that occurred within the intestinal tract, mainly in the rumen, were detrimental due to the impairment of copper absorption. However, there is clear evidence that thiomolybdate complexes are absorbed and have detrimental effects on copper metallo-enzymes (Mason 1988). Suttle (2002) contends that the thiomolybdate produced in the rumen will not be absorbed into the animal unless molybdenum is in molar excess of copper. However, this does not reflect current field evidence and scientific publications.

MATERIALS & METHODS
The University of Leeds has received >7500 blood samples from commercial dairy cattle over the past 8 years. These data are from 238 different veterinary practices submitting samples for commercial analysis. These samples were analysed for their plasma copper concentration, serum caeruloplasmin activity, erythrocyte superoxide dismutase activity and the TCA insoluble copper concentration (Kendall et al, 2001).

RESULTS

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Table 1. Differential distribution of blood variables for copper in all classes of dairy cattle

<table>
<thead>
<tr>
<th>% of samples (n=)</th>
<th>Plasma Cu µM/l</th>
<th>Serum caeruloplasmin activity mg/dl</th>
<th>Serum caeruloplasmin/plasma copper/ratio</th>
<th>TCA insoluble copper µM</th>
<th>SOD activity units/gHb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficient &lt;9.4</td>
<td>Normal 9.4 to 20</td>
<td>High &gt;20</td>
<td>Deficient &lt;15</td>
<td>Normal 15-45</td>
</tr>
<tr>
<td>Plasma Cu µM/l</td>
<td>2 (125)</td>
<td>94 (7421)</td>
<td>4 (309)</td>
<td>5 (363)</td>
<td>93 (7280)</td>
</tr>
<tr>
<td>Serum caeruloplasmin activity mg/dl</td>
<td></td>
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<tr>
<td>TCA insoluble copper µM</td>
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<td>SOD activity units/gHb</td>
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DISCUSSION
The plasma copper concentrations clearly show that there are very few animals that show hypocupraemia and this agrees with Suttle (2002). A small percentage do have an elevated plasma copper concentration and the vast majority of these have been shown to be due to an acute phase reaction as a result of infection or inflammation, as confirmed by follow-up contact with the farmer or vet. The distribution of caeruloplasmin activities closely mirrors the plasma copper distribution with a similar high percentage of the animals being in the normal range. Therefore there is little advantage to using caeruloplasmin over plasma copper as a single point determinant. Suttle (2002, 2003a) and Underwood & Suttle (1999) criticise the use of the
caeruloplasmin/plasma copper ratio, but as Mackenzie et al (1999) have shown, there is an effect of molybdenum on the activity of caeruloplasmin. As 22% of the cattle sampled (Table 1) have TCA insoluble copper (copper thiomolybdate) in their blood this is irrefutable evidence that this thiomolybdate is absorbed in significant quantities by dairy cattle. The use of superoxide dismutase activities can be helpful in diagnosing molybdenum toxicity, but it has to be recognised that this enzyme’s life equates to the life of the erythrocytes (21 to 27 weeks). However, changes in copper nutrition can be seen within days in plasma copper and caeruloplasmin. Therefore, relating plasma copper or caeruloplasmin directly to superoxide dismutase is incorrect unless the history of the animals’ copper intake is known. Mackenzie et al (1999), Williams et al (2001) and Williams (2004) demonstrated that the caeruloplasmin/plasma copper ratio was sensitive to distinguish between a molybdenum and sulphur challenge compared with an iron and sulphur challenge. Lambs supplemented with molybdenum/sulphur had significantly lower ratios. However, plasma copper levels were largely unaffected by treatment, or at the high levels of 10 mg/kg molybdenum plasma copper levels were significantly elevated. This alteration in the speciation of copper in plasma is of great interest as caeruloplasmin should account for 88% of plasma copper (Telfer et al 1996). Also Williams (2004) demonstrated that there was no effect of dietary molybdenum/sulphur and iron/sulphur on caeruloplasmin gene expression. Therefore, the difference in caeruloplasmin activity and caeruloplasmin/plasma copper ratio clearly indicates inhibition of enzyme activity and a systemic effect of molybdenum toxicity.

The serum caeruloplasmin/plasma copper ratio does differentiate between the effects of iron and molybdenum and can be used diagnostically to determine the presence of molybdenum toxicity in the animal. The major criticism by Suttle (2002) is that up to 20% of caeruloplasmin activity may be lost in the clotting process. The ratio of 1.5 or less allows for a 22% loss of activity whilst the marginal category (1.6 to 1.8) can be regarded as a grey area where the depression may be due to loss of activity in the clot, or to molybdenum toxicity, or a combination of both as the loss due to clotting is not consistent. Therefore, if we only diagnose those animals with ratios of 1.5 or less as suffering from molybdenum toxicity (clinical copper deficiency) there are 30% affected as compared to the 2% and 5% diagnosed by either plasma copper or caeruloplasmin respectively. If some within the marginal category of ratios are also affected by molybdenum toxicity (clinical copper deficiency) then the numbers could be over 50%. However, it has to be noted that the samples received may be biased, as these are from largely problem herds where the University of Leeds tests are regarded as a last resort investigation, all other lines of investigation having failed to find a cause for infertility.

The toxic effects of molybdenum in ruminant livestock are clearly evident from the literature and are likely to be mediated via the thiomolybdate complexes. Phillippo et al (1987) examined the effects of 5 mg/kg molybdenum and 800 mg/kg iron on growth and fertility of Hereford-Friesian heifers. Copper concentrations in plasma and liver were decreased in the supplemented heifers compared with the controls. However, it was only in the molybdenum supplemented heifers that effects on fertility were observed. Age at which puberty was achieved was delayed and liveweight was reduced in molybdenum supplemented animals compared with the controls or iron supplemented heifers. Also the pre-ovulatory LH surge was inhibited by molybdenum. Similar effects were reported in sheep by van Niekerk and van Niekerk (1989) and du Plessis et al (1999). Using cultured bovine ovarian cells, thiomolybdates were shown to depress steriodogenesis with decreased production of oestradiol (Kendall et al, 2003) and androstenedione (unpublished observation, Kendall and Campbell). These effects were partially ameliorated by the addition of copper to the culture medium which also contained bovine serum albumin viewed by Suttle (2003) as the main detoxifying agent. It is therefore clear that dietary molybdenum and thiomolybdates affect reproductive hormone production/ release. Further to this, Haywood et al
(2004) reported gross alterations in the pathology and function of the pituitary gland in ewes administered thiomolybdate parenterally. These cells showed a non-inflammatory atrophy or degeneration. Molybdenum accumulation in the pituitary was also reported in the thiomolybdate treated sheep. The sheep from this study that received the parenteral thiomolybdate became infertile and unthrifty. Williams (2004) reported that dietary molybdenum altered the pathology of the pituitary at 10 mg/kg DM. In the immunocytochemistry studies by Williams (2004), staining for ACTH in the pituitary glands of lambs supplemented with 2, 5 and 10 mg/kg DM molybdenum was more intense in a dose dependent manner, when compared with controls and lambs supplemented with iron. These findings suggest that dietary molybdenum at levels as low as 2.0 mg/kg and thiomolybdates are associated with a direct effect on the pituitary and endocrine system in ruminant animals. This effect appears to be in part due to a direct effect on the pituitary cells resulting in the prevention of hormone release. The mechanism of this has not yet been established.

However, there is currently interest in the role of the copper dependent enzyme, peptidylglycine α-amidating monoxygenase (PAM) (Stevenson et al, 2003). Activity of PAM is essential for the secretion of numerous peptides, hormones and neurotransmitters from cells. Inhibition of PAM activity has been demonstrated by the removal of copper by the chelator disulfiram (Bolkenius and Ganzhorn, 1998) and by preventing the supply of copper to it by mutation in the copper transport protein ATP7a (Stevenson et al, 2003). Therefore it can be concluded that some of the clinical effects of molybdenum toxicity may be mediated via inhibition of PAM activity within the pituitary.

Infertility in the British dairy industry has a dramatic effect on performance and economic viability. Infertility is clearly a multifactorial problem. A major factor in fertility is poor energy utilisation. Work by Frank et al (2002) has shown a significant depression in the activity of the enzyme cytochrome c oxidase in Swedish moose due to molybdenum toxicity. Cytochrome c oxidase is an important enzyme responsible for oxidative phosphorylation and energy production in the cell. Recent press reports (Porter 2004) show that on a farm diagnosed as having a serious molybdenum toxicity (clinical copper deficiency) correction of the problem with ruminal copper supplementation has not only improved fertility from a conception rate to first service of 8% to a peak of 90%, but also raised milk yield from 5,557 litres to 8,073 litres over a three year period. (Porter 2004). It is clear that there is a depression of energy utilisation caused by molybdenum toxicity with a depression in cytochrome c oxidase activity (Frank et al, 2002) being the likely cause. The review of published papers presented above clearly shows that molybdenum toxicity does impair the fertility of ruminants. This problem is underestimated in cattle within the UK. The recent paper by Black and French (2004) showed fertility responses to copper supplementation in three dairy herds where the measured dietary molybdenum intakes were below 2.0 mg/kg and plasma copper concentrations were within the normal range.

Mackenzie et al (2001) also demonstrated amelioration of sub-fertility by copper supplementation when the herbage molybdenum level was 2.3 mg/kg. These effects on fertility included a decrease in the number of inseminations to confirmed conception and a reduction in the calving interval (Table 2).

| Table 2. Effect of Copper supplementation on the fertility of dairy cattle. |
|-----------------|------------|----------|----------|-------|
|                | Control    | Supplemented | SE mean | Sig   |
| No of inseminations | 2.5       | 1.7       | 0.16     | P<0.01|
| Calving Interval (days) | 397       | 342       | 9.2      | P<0.05|
| Calving to conception (days) | 117       | 95        | 9.3      | NS    |

Mackenzie et al 2001
These values of molybdenum intakes reported by Mackenzie et al (2001) and Black and French (2004) are levels previously thought to be insignificant. However, iron exacerbates this problem and major sources of dietary iron will be from grazing, borehole water, inappropriate mineral supplements and soil contamination of forage. Although the work by Phillippo et al (1987) did not show additional effects of iron supplemented along with molybdenum, it is well recognised that winter grazed ewes’ ingestion of soil precipitated clinical copper deficiency in the form of swayback. The interactions between iron, molybdenum and sulphur require further investigation in dairy cattle. Clearly not all infertility in cattle is due to molybdenum toxicity and inappropriate, excessive supplementation of copper will predispose the animal to copper toxicity. The information provided here shows that correct diagnosis of molybdenum toxicity is essential. Once diagnosed, treatment requires a careful assessment of the challenge on a farm basis. A reduction in iron intake will be beneficial, but is not always practicable.

Thiomolybdates are produced within the rumen and if these complexes do not bind to copper in the rumen they will be absorbed by the animal as shown by the presence of TCA insoluble copper in blood (Table 1). Therefore, where molybdenum toxicity is diagnosed, copper supplementation is required to prevent thiomolybdate absorption from the rumen rather than to meet a metabolic demand for copper. Clearly, Black and French (2004) demonstrated that parentally administered copper, or oral copper oxide, was not as effective at alleviating clinical symptoms when compared with copper delivered and available within the rumen. It can be concluded that this copper within the rumen has been sacrificed to prevent molybdenum toxicity and not as a supply of copper as an essential trace element that has to be absorbed into the body.

**CONCLUSION**
The work reported and reviewed clearly establishes that molybdenum toxicity (clinical copper deficiency) occurs in ruminants. The molybdenum toxicity that occurs is independent of the copper status of the animal and is a result of the interaction between iron, molybdenum, sulphur and copper occurring in the rumen. This allows toxic thiomolybdate molecules to be absorbed into the body and “poison” critical copper enzymes leading to a depression in fertility and energy utilisation. Molybdenum toxicity can be cured by a combination of reducing the iron intake of the animal and copper supplementation. However, the effect is in the rumen and it is necessary that the copper supplemented to the animal should be available in the rumen and not supplied primarily as an absorbable source. Diagnosis and subsequent prevention of molybdenum toxicity (clinical copper deficiency) by correct supplementation with copper will improve fertility and production. However, copper supplementation for absorption into the animal without diagnosis will not be effective and can result in an excessive intake of the element and subsequent danger of copper toxicity. Molybdenum toxicity is a real condition and the full extent of its impact on the UK dairy herd has not yet been recognised.

**REFERENCES**


