

## A COMPARISON OF THE EFFICACY OF PROPRIETARY PRODUCTS IN THE TREATMENT OF MOLYBDENUM INDUCED COPPER DEFICIENCY.

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### INTRODUCTION

Clinical copper deficiency has been described in ruminant animals with various visual symptoms being observed. One such visual symptom is the alteration of hair/ wool pigmentation. The cause of clinical copper deficiency has until recently been defined as a lack of copper that is available for absorption into the animal, with the antagonistic interactions of iron / sulphur and molybdenum / sulphur acting to reduce the amount of 'available' copper. However, work by Phillip, Humphries, Atkinson, and Henderson (1987) has shown that clinical copper deficiency is due to a toxic effect of molybdenum rather than a lack of copper for metabolic function. Copper in excess of that required for essential metabolic function ( $< 1$  mg/ kg DM, Zervas, 1983) is used as an agent to react with the toxic molybdenum/ sulphur compounds (thiomolybdates) to render them inactive and harmless to metabolic function. The efficacy of copper supplementation of ruminant animals is the ability to correct the symptoms of clinical copper deficiency and should not be judged by the supplement's ability to raise the copper content of the body. Liver copper and total blood copper are still used alone as indicators of copper status, these do not take any account of the correction of the symptoms of clinical copper deficiency. The experiment described here uses a visual symptom (alteration in wool pigmentation) as one of an extensive set of copper status indicators to assess the efficacy of a variety of proprietary copper supplements.

### MATERIALS AND METHODS

Twelve hebridean lambs (black woolled) were housed in two pens of six. They were fed a concentrate diet low in copper (3 mg/kg), high in iron (1217 mg/ kg) and molybdenum (3.6 mg/ kg) with moderate sulphur (2 g/ kg) with *ad libitum* access to barley straw (3 mg Cu/ kg, 997 mg Fe/ kg, 0.7 mg Mo/ kg and 1 g S/ kg). Additional sulphur (0.4 g/ sheep day) and molybdenum (1.2 mg/ sheep day) were added to the feed.

The sheep were sheared at the start of the experiment and a patch approximately 10 cm x 10 cm kept trimmed to enable easy comparison of emerging wool colour to existing wool. Once the sheep were 'copper deficient' and exhibiting white wool they received the ewe dose of one of five proprietary treatments or were maintained untreated as controls. The treatments were 2ml of a veterinary surgeon prepared drench (40 mg Cu), 20 ml of Liquithrive (Agri-Lloyd) a copper chelate drench (80 mg Cu), a 33 g Cosecure (Telsol Ltd) soluble glass bolus (4.4 g Cu), a 4 g capsule of Copporal (Beecham) copper oxide needles (3.2 g Cu) and 2 ml of Cuvine (C-vet) copper heptonate injection i.m. (25 mg Cu).

Copper status was assessed by plasma copper concentration (PICu), serum caeruloplasmin activity (CP), erythrocyte superoxide dismutase activity (SOD), trichloroacetic acid soluble copper in the plasma (TCA), serum amine oxidase activity (AMOX) and the ratio between CP and PICu (CP/PICu) using the methods of Mackenzie, Illingworth, Jackson, and Telfer, (1997) and Mulryan and Mason (1992).

### DISCUSSION OF RESULTS

The control sheep were the sheep in each pen with the best copper status (judged by wool pigmentation) at time of treatment, and this has dulled the response of the treatments when compared to the controls. Wool colour especially illustrates this as in one of the control sheep the wool did not turn white due to alterations in pigmentation but in fact ceased growth altogether, became loose and fell out. The treatments were allocated in reverse order of expected duration of

efficacy with the two oral liquids (drench and chelate) being administered to the next two highest copper status sheep, followed by the injection, needles and bolus.

The plasma copper concentrations (Figure 1) were in the normal range for most of the sheep after treatment, although the control sheep were not as low as may have been expected (normal to marginal range) based on assessing their clinical symptoms. The other sheep were occasionally classified as marginal except for one of the drench treated sheep after week 7, which was deficient. Three sheep (injection, chelate and needles) had plasma copper concentrations at levels where toxicity would be a concern ( $>23 \mu\text{M}$ ). The TCA soluble copper (Figure 2) paints a different picture with the control and drench treated sheep having deficient amounts of copper when the TCA insoluble copper tetrathiomolybdate was precipitated off. The chelate was only able to increase the TCA soluble copper from marginal on three samplings and for one sheep only. The injection gave a good initial boost but was drifting back towards marginal as the experiment progressed. The needles gave a better response than did the injection which was sustained throughout for one of the two sheep. The bolus maintained an adequate TCA soluble copper concentration throughout the experiment. The TCA insoluble fractions illustrate how the different treatments are working. A high TCA insoluble copper fraction indicates a large amount of copper tetrathiomolybdate (Cu-TTM) present in the blood therefore suggesting any effective copper treatment to be by providing copper to react with absorbed TTM in the blood thus preventing the stripping of metabolic copper. A low TCA insoluble fraction indicates that there is little or no Cu-TTM in the blood due to either there being no uptake of TTM into the blood (i.e. by chelating copper to form CU-TTM in the rumen which is not absorbed by the sheep) or that there is no copper available in the blood (plasma amino acid copper chelate) to react with the absorbed TTM and hence the TTM will react with sources of metabolic copper and depress enzyme activities (illustrated by the CP/ PICu ratio) (Mackenzie et al., 1997). Figure 3 shows the TCA insoluble fractions, and it can be seen that both bolus treated sheep were maintaining low TCA insoluble fractions indicating a prevention of the uptake of free TTM into the blood. This effect was also shown by one of the needle treated sheep. The caeruloplasmin activities (Figure 4) were mainly normal ( $>15 \text{ mg/dl}$ ) apart from one of the control and both drench treated sheep. The injection and needles gave the largest increase in CP. The bolus and injection maintained CP in the normal range throughout whilst the chelate and needles were both deficient in one sheep on one sampling. The CP/ PICu ratio (Figure 5) can be used to give an indication of free tetrathiomolybdate in the blood. The ratio was lowest for the drench treated sheep and the controls. The chelate was variable with one of the sheep having a very poor response whilst the other showed a ratio response for most of the experimental period. The injection had the greatest immediate effect, however this effect was lost by week 5. The needles and bolus were the best treatments for this parameter, although the needles seemed to be very effective in one sheep and poorer in the other, losing the effect after week 5. Serum amine oxidase activities (Figure 6) were low for the control sheep and the second chelate treated sheep. The superoxide dismutase activities (Figure 7) have a longer half-life than the other parameters and reflect the copper status over the previous six weeks. In terms of SOD the needles gave the quickest response which was maintained until the end. The bolus and injection were lower initially although the response was sustained throughout. The chelate had a three week transitory response, whilst the drench only had a week response. The wool colour (Figure 8), an analysis of a clinical symptom (alteration of wool pigmentation), was not maintained black by either the chelate or the drench. The bolus had the quickest response in wool colour, which was maintained throughout the rest of the trial, as was the injection and needle response. The control sheep that maintained black wool throughout had in fact ceased wool growth and this wool was loose, with bare patches occurring.

The drench, chelate and needles primarily rely on the absorption of the copper in order to exert a beneficial effect. The absorbed copper (and copper from injection) then acts probably as plasma amino acid bound copper, allowing the TTM to strip copper from the amino acids in preference to stripping the copper from its metabolically active roles (copper dependant enzymes etc.). The

longevity of response is dependant on the ability of the treatment to resupply copper to the plasma amino acids and hence release enough amino acid bound copper to counteract the free TTM. The needles also maintain longevity by the nature of the slow dissolution of the oxidised copper. The bolus works in a different manner, with the release of copper into the rumen which binds to the free TTM to form Cu-TTM which is not absorbed and is egested in the faeces, thus giving the animal a lowered copper requirement as there is little free TTM requiring detoxification in the blood of the animal.

## CONCLUSIONS

Over the 10-week duration of the trial, the bolus gave the best maintenance of copper status and correction of clinical copper deficiency by preventing uptake of free tetrathiomolybdate into the sheep. The needles and injection also corrected the clinical copper deficiency, with the injection increasing the plasma copper and caeruloplasmin more, whilst the needles were better when the CP/PICu ratio and TCA insoluble copper were assessed. The chelate was better than the drench for copper status although neither could sustain the correction of the clinical deficiency.

## REFERENCES

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