Liver copper concentration dynamics with different methods of injectable copper supplementation in New Zealand dairy cows

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Objective

To determine if the temporal pattern of liver copper concentrations differs between three copper injectable supplementation methods and a negative control group.

Background

There is a lack of clarity on how much copper is supplied to the liver and for how long this persists following parenteral copper supplementation. It is therefore challenging for veterinarians and farmers to predict the likely response from supplementation.

Parenteral administration of copper is frequently used to treat or prevent hypocuprosis and is often chosen for practical reasons or to avoid gastrointestinal antagonism which can occur with oral delivery mechanisms. However, parenteral administration of copper is potentially more dangerous and can lead to death by hepatotoxicity if given in overdose (Bohman *et al.* 1987, Bulgin *et al.* 1986, Galey *et al.* 1991).

In cattle, copper is stored in the liver, thus, to identify the effect of copper supplementation requires multiple measures of liver copper concentration over time using percutaneous liver biopsy.

Materials and methods

The study was undertaken on two commercial dairy farms located in North Canterbury, New Zealand. The study enrolled 80 dairy cows in mid-lactation, with cows evenly split into two age groups: 3-5 and 6-10 years. Cows were randomly allocated to one of four treatment groups: Group 1: 100mg copper as calcium copper EDTA (2ml Copperguard, Virbac, NZ); Group 2: 200mg copper as calcium copper EDTA (4ml Copperguard, Virbac, NZ); Group 3: 75mg copper as disodium copper EDTA combined with selenium, zinc, and manganese (5ml MultiMin, Virbac, NZ); and Group 4: no supplementation (negative control). Each treatment group contained 20 animals, with ten animals per age group.

Liver biopsies were collected from each cow on day -7 (7 days prior to treatment) with copper treatments given on day 0. Each cow was further sampled over six liver biopsy events: day 3, day 14, day 28, day 42, day 56, day 70. At each liver biopsy event, all animals received NSAID (Ketomax, AgriHealth NZ) IM.

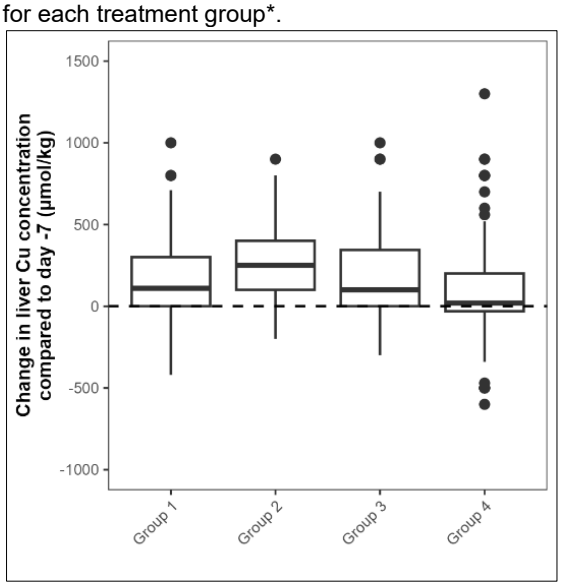
The study was designed to detect a 200µmol/kg increase in liver copper concentration, or difference between treatment groups. Data was collated and analysed to assess changes in liver copper concentration at each biopsy event compared to day -7 (µmol/kg). A mixed multivariable linear regression model was constructed to determine the effect of treatment group on the change in liver copper concentration compared to pre-treatment concentrations. It accounted for repeated measurements taken from each cow and interactions with time and farm. Predicted changes in liver copper concentration were derived from the final model and plotted.

Results

From the 80 cows enrolled, 77 were present on day 70 (one cow was excluded due to sickness, and two cows died). Treatment groups were balanced for age, plasma copper concentration, and day -7 liver copper concentration. Median pre-treatment liver copper concentration was 1,200µmol/kg (IQR 888, 1,500). There was wide variation in day -7 liver copper concentration across animals on each farm (p=0.008), with ranges of 530-2,000 and 200-1,900µmol/kg on Farms 1 and 2 respectively. Before adjusting for other variables, median changes in liver copper concentration after supplementation were significantly higher for Farm 1 (310, IQR = 195-500µmol/kg) than for Farm 2 (100, IQR = -30-200µmol/kg) (p<0.001).

When comparing the overall effect of treatments across both farms, there was a significant unadjusted difference between treatment groups (Figure 1). Unadjusted median changes in liver copper per treatment group when compared to day -7 were highest for Group 2 (250µmol/kg, IQR = 100-400), and similar for Group 1 and Group 3 respectively (110µmol/kg, IQR = 0-300; and 100µmol/kg, IQR = 0-345) (p<0.001).

Figure 1. Distribution of change in liver copper concentration compared to day -7, across the study period for each treatment group*.



Boxes extend from the 25th to the 75th percentiles, with a horizontal line at the median. Whiskers extend to values no more than 1.5 times the interquartile range. Data beyond the end of the whiskers are deemed outliers and are plotted individually.

*Group 1: 100mg copper as calcium copper EDTA; Group 2: 200mg copper as calcium copper EDTA Group 3: 75mg copper as disodium copper EDTA combined with selenium, zinc, and manganese; Group 4: no supplementation (negative control).

A multivariable model showed a three-way interaction between treatment group, study day, and farm, when compared to liver copper concentration at day -7. Age was not associated with a change in liver copper concentration. Predicted changes in liver copper concentration over time compared to day -7 per treatment group and by farm are shown in Figure 2. Significant differences were found for Group 2 compared to Groups 1, 3, and 4 but only on Farm 2 and on certain days (Table 1).

Table 1. Pairwise contrasts from the final multivariable mixed linear regression model of the change in liver copper concentration compared to day -7 for Farm 2. Only significant contrasts at the p<0.05 level are shown.

Study day	Contrast	Estimated difference (µmol/kg)	95% CI (µmol/kg)	p-value
3	Control - 200mg Ca Cu EDTA	252	24-481	0.025
14	Control - 200mg Ca Cu EDTA	325	97-554	0.002
14	100mg Ca Cu EDTA - 200mg Ca Cu EDTA	233	7-460	0.041
14	200mg Ca Cu EDTA - 75mg Na Cu EDTA	248	22-474	0.026
28	Control - 200mg Ca Cu EDTA	287	51-523	0.011
28	100mg Ca Cu EDTA - 200mg Ca Cu EDTA	267	25-509	0.024
42	Control - 200mg Ca Cu EDTA	243	5-482	0.043

Change in liver copper concentration was negatively associated with day -7 concentration and for each 10µmol/kg increase in liver copper concentration at day -7, the change in liver copper at subsequent biopsy events was predicted to reduce by 1µmol/kg. It was noted 57% of the variation in change in liver copper concentration occurred at the cow level and 43% at the biopsy level.

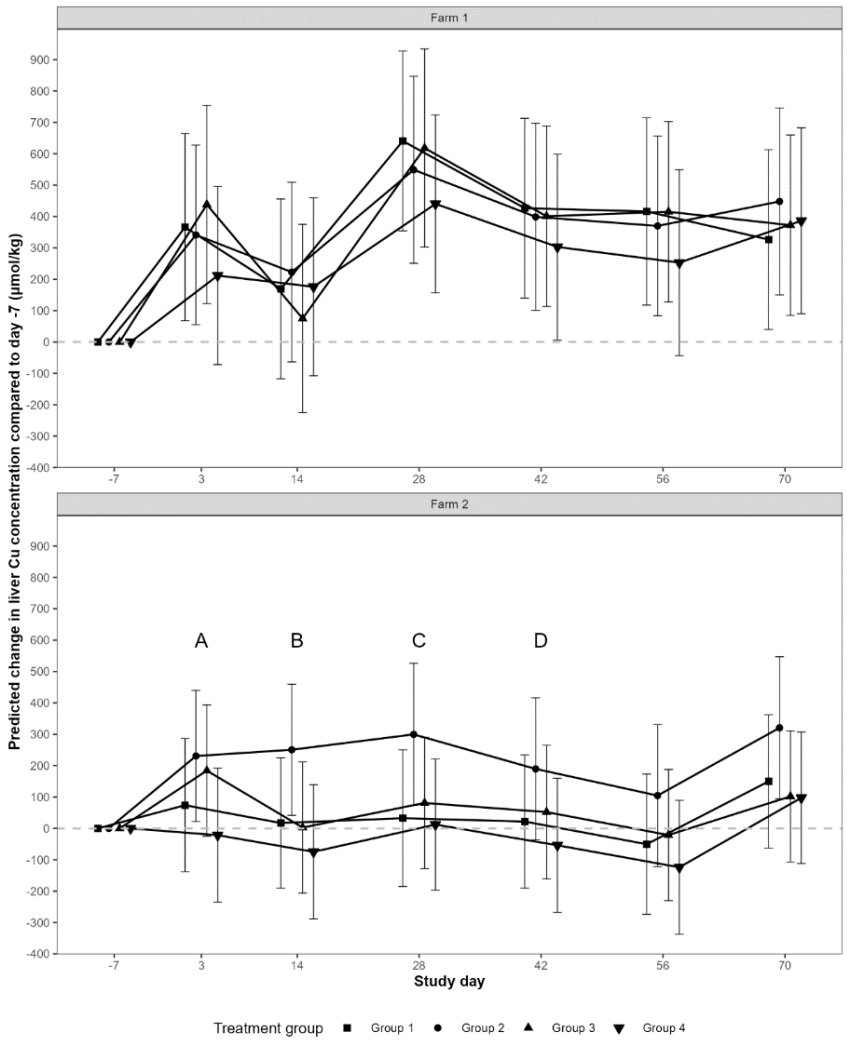


Figure 2. Predicted change in liver copper concentration compared to day -7, at each biopsy event (study day), for each treatment group and by farm. Treatment was administered at day 0. Vertical lines represent 95% confidence intervals.

A = significant difference between groups 2 and 4; B = significant difference between group 2 and groups 1 and 3; C = significant difference between group 2 and groups 1 and 4; D = significant difference between groups 2 and 4.

*Group 1: 100mg copper as calcium copper EDTA; Group 2: 200mg copper as calcium copper EDTA Group 3: 75mg copper as disodium copper EDTA combined with selenium, zinc, and manganese; Group 4: no supplementation (negative control).

Discussion

Overall, cows that started with lower liver copper experienced greater increases in stored copper after treatment. These results suggest there are diminishing returns from copper supplementation with increasing baseline liver concentrations. This supports the findings of Balemi *et al.* (2010) who also studied responses to supplementation in copper sufficient animals and reported initial liver copper concentrations can significantly affect the rate of accumulation of copper among cows. Balemi *et al.* (2010) showed cows with an initial liver copper concentration <1,100µmol/kg reported an increase of 4.1µmol/kg fresh tissue/day, whereas rates were variable or even negative when initial liver copper concentration was >1,100µmol/kg. Therefore, knowing the liver copper status on a farm prior to treatment will help veterinarians judge the value of copper supplementation.

There was a lack of consistent response to treatments across the two study farms, highlighting individual farm variability in responses to parenteral copper supplementation. The final model showed a statistical difference over time only on one farm for the 200mg calcium copper EDTA group, where the predicted increase in liver copper following a single injection of 200mg calcium copper EDTA was 325µmol/kg. The duration of persistence was found to be significant for 42 days after treatment where liver copper was 250µmol/kg higher than controls. These findings correlate with Hawkins (2014) who reported a 200mg copper injection was effective in increasing liver copper concentration by around 300µmol/kg for at least 42 days. These results also suggest a single injection of 200mg copper is unlikely to cause negative effects unless animals are already close to a toxic threshold (since the increases in concentration were not large enough to place cows near the toxic range) but highlights the precautions necessary if recommending repeated higher doses of injectable copper products.

While 100mg calcium copper EDTA and 75mg disodium copper EDTA groups showed similar unadjusted elevations in liver copper concentrations compared to baseline, (presumably due to the products containing a similar concentration of copper), these increases in liver copper concentration did not achieve statistical significance when compared to untreated controls. Sample size may have played a role as the study was designed to detect a 200µmol/kg increase between treatment groups. Balemi (2010) also reported no significant response within copper sufficient animals to an injection of 100mg copper. This study illustrates that supplementing with a single injection of 100mg copper may not provide sufficient elevation in liver copper concentration and establishing the need for copper supplementation may be more relevant.

Conclusion

Among cows that are not copper deficient, response to copper supplementation depended on farm and changed across time for each treatment. Only 200mg calcium copper EDTA significantly increased liver copper concentration to a peak of 325µmol/kg on day 14 compared to pre-treatment baseline, and this occurred only on one farm and at certain time points. A single injection of 200mg calcium copper EDTA was predicted to elevate liver copper concentration for at least 42 days.

References

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This study was conducted under approval by AgResearch Ruakura Animal Ethics Committee, Hamilton, NZ (application number 727).

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