

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**AN EVALUATION OF THE EFFICACY OF  
ORALLY ADMINISTERED COPPER GLYCINE  
COMPLEX, COPPER AMINO ACID CHELATE,  
COPPER SULPHATE, A COPPER OXIDE WIRE  
PARTICLE BOLUS AND A COPPER EDETATE  
INJECTION IN NEW ZEALAND DAIRY COWS**



A thesis presented in partial fulfillment of the requirements for the degree of

Masters in  
Animal Science

Massey University, Palmerston North, New Zealand.

Shaun Christopher Balemi

2008

## ABSTRACT

This thesis set out to examine the difference in efficacy of the most commonly used copper supplements in New Zealand dairy herds. There is limited information on copper supplementation in New Zealand dairy cattle in the area of chelated (organic) versus sulphated (inorganic) supplements and this study was designed to provide more information to the New Zealand dairy industry.

Sixty non-pregnant mixed age Friesian dairy cows, on the basis of liver copper concentrations, were randomized into 6 groups of 10 animals so that each group had the same mean liver copper concentration. The treatments were Group 1, non-supplemented control; Group 2, 150mg copper/day as copper glycine chelate drench; Group 3, 150mg copper/day as copper amino acid chelate drench; Group 4, 150mg copper/day as copper sulphate drench; Group 5, 20g copper oxide wire particles administered as a bolus and Group 6, 100mg of copper, as calcium copper edetate, administered as a subcutaneous injection on days 1 and 58. The duration of the study was 116 days and the cows were fed baleage, with limited access to pasture. On days -5, 14, 28, 58, 86, and 116 after supplementation, liver samples were obtained by a biopsy technique and blood from the coccygeal vein for copper determinations.

The mean initial copper concentration in the liver of the cows used in this study was 827 (SE 109)  $\mu\text{mol/kg}$  fresh weight (FW). The mean liver copper concentration of the cows in the control group decreased significantly ( $P < 0.05$ ), from 827 (SE 109)  $\mu\text{mol/kg}$  FW on day 1 to 554 (SE 114)  $\mu\text{mol/kg}$  FW on day 116. Over days 58 to 116 the mean liver copper concentration of the copper glycine chelate, copper amino acid chelate, and copper sulphate groups were significantly ( $P < 0.05$ ) greater than the non-supplemented control group. The combined means over the 6 sampling events showed that the group supplemented with the copper glycine chelate had significantly ( $P < 0.05$ ) greater liver copper concentration than the group supplemented with copper sulphate (1064 versus 910  $\mu\text{mol/kg}$  FW). The mean liver copper concentrations of the group which received the copper oxide wire particle boluses were consistently greater than the control group;

however a significant difference was only achieved at the day 58 sampling. The group injected with copper edetate achieved a significant rise in liver copper concentration on day 86 after being injected on day 58. However, when the group was injected at day 1 no significant rise was achieved at day 14, 28, or 58. The copper supplements had no effect on serum copper concentrations. Despite the large variation (SE 109) in initial liver copper status between the cows, this did not influence the amount of copper stored in the liver regardless of the copper supplement used. The data was analysed in two groups, cows with lower liver copper (553  $\mu\text{mol/kg FW}$ ), and cows with higher liver copper (1050  $\mu\text{mol/kg FW}$ ), and there was no difference between the two groups in response to the copper treatments.

The initial liver copper concentration of the cows was high. A copper intake of 150mg copper/day was effective in increasing the copper concentration of the liver of dry non-pregnant New Zealand dairy cows. As an oral supplement, copper glycine chelate was more effective in increasing liver copper concentrations than copper sulphate. Overall the oral supplements (copper glycine, copper amino acid chelate, and copper sulphate) were more effective in increasing liver copper concentration than the copper oxide wire particle bolus and the twice given 2ml copper edetate injections. The copper oxide wire particle bolus maintained liver copper concentrations at 843 $\mu\text{mol/kg FW}$  which is an adequate liver copper concentration. Therefore in this situation where liver copper concentrations were adequate prior to supplementation the bolus did provide enough copper. This study indicated that in order to maintain liver copper concentration in dry non-pregnant New Zealand dairy cows, on a low copper diet, a 2ml injection may have to be given every 45 days.

# ACKNOWLEDGEMENTS

I would like to thank Prof Dave West and Dr Neville Grace for their commitment to directing and aiding me in the completion of this thesis, without their support the writing of this thesis would not have been possible.

I would like to thank my Father and Mother for the support, both moral and financial, which they have freely given.

I would like to thank Robin Whitson, Stefan Smith, various 5<sup>th</sup> year vet students, and the rest of the LATU staff for the management of the animals used in the trial, for direction and commitment to the drenching, and for their help on the sampling days.

I would like to thank the Taupo Animal Welfare Health and Veterinary Society Inc, Agvance Marketing Ltd, and Massey University for the financial assistance they generously provided making this research project possible.

I would like to thank Nicolas Lopez-Villalobos for his direction in the statistical analysis of the data.

I would like to thank all the postgrads on the 3<sup>rd</sup> floor for their willingness to give help and answer questions.

Lastly I would like to thank the Lord God for His provision, favour, and blessing, for without Him none of this would have been possible.

# TABLE OF CONTENTS

|  |           |
|--|-----------|
| <b>Abstract.....</b>   | <b>2</b>  |
| <b>Acknowledgements.....</b>   | <b>4</b>  |
| <b>Table of Contents.....</b>  | <b>5</b>  |
| <b>List of tables.....</b>   | <b>6</b>  |
| <b>List of figures.....</b>  | <b>8</b>  |
| <b>Introduction.....</b>   | <b>10</b> |
| <br>   |           |
| <b>Chapter 1: Literature Review.....</b>   | <b>13</b> |
| Functions of copper in ruminants.....  | 13        |
| Metabolism of copper in ruminants.....   | 18        |
| Clinical manifestations of copper deficiency in ruminants.....                             | 33        |
| Diagnosis of copper deficiency in ruminants.....   | 41        |
| Copper requirements for ruminants.....   | 47        |
| Supplementation of copper in ruminants and the different forms of copper<br>available..... | 50        |
| <br>   |           |
| <b>Chapter 2: Materials &amp; Methods.....</b>   | <b>62</b> |
| <b>Chapter 3: Results.....</b>   | <b>71</b> |
| <b>Chapter 4: Discussion.....</b>  | <b>80</b> |
| <b>References.....</b>   | <b>86</b> |
| <b>Appendix.....</b>   | <b>94</b> |

# LIST OF TABLES

|  |       |
|--|-------|
| <b>Table 1.</b> The major copper dependant enzymes and there functions in the mammalian body .....   | 14    |
| <b>Table 2.</b> Estimates on the percentage of copper absorbed by Scottish Blackface ewes from seven major forage types (Underwood & Suttle, 1999).....  | 21    |
| <b>Table 3.</b> The effects of high iron (Fe) and molybdenum (Mo) levels on cattle liver and plasma copper levels and the effects on cattle reproductive performance (Phillippo et al., 1987).....   | 24    |
| <b>Table 4.</b> Growth response in cattle to copper supplementation when compared to mean serum and liver copper levels derived from overseas studies (Ellison, 1992).....   | 34    |
| <b>Table 5.</b> The effect of copper and molybdenum supplementation on live-weight gain in cattle and sheep (adapted from Phillippo 1983).....   | 35    |
| <b>Table 6.</b> The tissue reference ranges used diagnose copper deficiency in cattle, sheep and deer (Grace, personal communication, 2008).....   | 41    |
| <b>Table 7.</b> Dietary copper requirements for dairy cattle (ARC, 1980).....  | 49    |
| <b>Table 8.</b> Summary of the peer-reviewed data on the efficacy of chelated copper supplements in livestock.....   | 55-56 |
| <b>Table 9.</b> The amounts of each copper treatment given to the cow (according to its treatment group) over the duration of the trial assuming the CuO Bolus would be spent after the 116 day trial period, and the elemental copper percentage of each copper treatment.....  | 65    |
| <b>Table 10.</b> Results of the pasture and baleage analysis for a full range of minerals, performed by Gribbles Analytical Laboratory, Hamilton, NZ.....  | 71    |
| <b>Table 11.</b> The overall changes in the mean ( $\pm$ standard error) liver copper concentration ( $\mu\text{mol/kg}$ fresh weight) of dairy cows not supplemented or supplemented with copper over a period of 116 days where each of cows over each of the sampling periods was analysed to reduce variation..... | 73    |

**Table 12.** The mean ( $\pm$  standard error) initial and final liver copper concentrations ( $\mu\text{mol/kg FW}$ ) in dairy cows not supplemented, or supplemented, with various copper products over a period of 116 days, and the estimated daily change in liver concentrations.....75

**Table 13.** The mean ( $\pm$ standard error) change in liver copper concentration within the groups, between the cows with high ( $>600 \mu\text{mol/kg FW}$ ) initial liver copper concentration and low ( $<600 \mu\text{mol/kg FW}$ ) initial liver Copper concentration, when not supplemented or supplemented with 150 mg Cu/day in three different forms over a period of 116 days.....76



# LIST OF FIGURES

- Figure 1.** The processes involved in copper metabolism in a 50kg Romney ewe at maintenance, showing where copper enters the animal in the diet, a proportion of that copper is absorbed into the blood and is then circulated around the body with a proportion of that copper being used by the body, used for production, stored in the liver, and excreted via the urine (Lee & Grace, 1997).....18
- Figure 2.** Severely copper deficient fawn shown skeletal abnormalities where the hocks are touching (McDowell, 2003)..... 36
- Figure 3.** Graph of the changes in copper status in the liver, blood, and sites requiring copper for function over a period of copper depletion, and the point where sub-clinical and clinical copper deficiency occurs (Minatel & Carfagnini, 2002).....44
- Figure 4.** Diagram showing the correlation between liver copper stores ( $\mu\text{mol/kg FW}$ ) and blood plasma copper concentrations ( $\mu\text{mol/L}$ ) in cattle (Legleiter & Spears, 2008).....45
- Figure 5.** Factorial model for the factors involved in the determination of copper (mg) requirements for dairy cows. (\*where molybdenum concentration is 1 mg/kg DM), (\*\*where DM intake is 17 kg). (ARC, 1980; Grace, 2004).....48
- Figure 6.** Pasture copper (copper) concentrations per kg dry matter (DM) after no treatment, topdressing with 6 kg or 12 kg  $\text{CuSO}_4$ /ha in mid-March 2001–2002 season (Grace et al., 2004).....59
- Figure 7.** Mean (a) serum, and (b) liver copper (Cu) concentrations of weaner hinds ( $n=11$ ) grazing untreated pasture and pastures top-dressed with 6 kg or 12 kg copper sulphate/ha in mid-March in Year 1. Standard errors are represented by vertical bars (Grace et al., 2004).....60
- Figure 8.** Changes in the mean ( $\pm$  standard error) liver copper concentration of dairy cows not supplemented, or supplemented, with Cu glycinate chelate, Cu amino acid chelate and Cu sulphate, over a period of 116 days.....73
- Figure 9.** Changes in the mean ( $\pm$  standard error) liver copper concentration of dairy cows not supplemented or supplemented with, 20g CuO wire particles at Day 1 or two Cu injections (100 mg) administered at Days 0 (1<sup>st</sup>) and day 58 (2<sup>nd</sup>), over a period of 116 days.....74

**Figure 10.** Changes in liver copper concentration in non supplemented dairy cows with either an initially high (>600 µmol/kg FW) or low (<600 µmol/kg FW) liver copper concentration of 116 days.....76

**Figure 11.** Changes in mean liver copper concentrations of dairy cows with either an initially high (>600 µmol/kg FW) or low (<600 µmol/kg FW) liver copper concentration and then supplemented with 150 mg of copper as Cu glycinate, Cu amino or Cu sulphate over a period of 116 days.....77

**Figure 12.** Changes in the mean (± standard error) liver copper concentration of dairy cows not supplemented, or supplemented, with Cu glycinate chelate, Cu amino acid chelate and Cu sulphate, CuO bolus, and a Cu injection over a period of 116 days.....78