SOME ASPECTS OF COPPER TOXICITY IN SHEEP GRAZING NEW ZEALAND PASTURES

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy in Veterinary Science
at Massey University.

Bruce Campbell Farquharson
B.V.Sc.(Queensland)

December 1984

ABSTRACT

Several authors have suggested a role for different predisposing factors in cases of copper toxicity, but any association between these factors and biochemical changes in the sheep have not been studied. Therefore the objective of this study was to look for the changes that occurred in sheep treated regularly with a parenteral form of copper. After identifying some of these parameters, further sheep were subjected to stressors that may be encountered in normal farming practice and the changes in response to copper treatment were compared to those of sheep not subjected to that stessor but treated with the same dose of copper.

Eleven Romney rams were regularly monitored to measure any changes that occurred in certain biochemical parameters when the sheep received a weekly subcutaneous injection of 50mg of copper calcium edetate. The biochemical parameters included serum levels of sorbitol dehydrogenase (SDH) and serum glutamate oxaloacetate transaminase (SGOT), blood copper concentration, and wool copper content. At fortnightly intervals, liver biopsy samples were collected for an estimation of the copper content and for a histopathological examination.

It was found that the activity of SDH and SGOT in the serum became elevated as the liver accumulation of copper increased. SDH levels were the first to change and became elevated up to six weeks earlier than SGOT. Although the pathological changes in the hepatocytes developed progressively, their severity and extent did not have a direct relationship to liver copper content. There was no significant change in the copper content of either blood or of wool during the period that the liver copper content increased.

The determination of indicators of early changes in cases of toxicity allowed stressors to be superimposed on copper therapy to assess whether such stressors might influence copper toxicity. The sheep involved received 3mg Cu/kg bodyweight as copper calcium edetate on the premise that sheep of low bodyweight might receive a double injection of the currently recommended dose of 50 mg per sheep. The same biochemical parameters of SDH and SGOT activity, blood copper concentration and liver copper content were used to assess the potentiating effects on copper toxicity of sheep first treated with copper calcium edetate parenterally and then subjected to various stressors. For this purpose 56 sheep, with additional control animals where appropriate, were divided into their respective groups. The stressors included dehydration by removing 25% of blood volume, starvation by fasting for 48 hours, exposure to cold ($5^{o}C$) for 5 days, and exposure to heat ($40^{o}C$) for 5 days. Other sheep were either immersed in an organophosphate insecticide, or treated with thiabendazole anthelmintic at a dose rate of 100 mg/kg bodyweight. Pregnant sheep, and others heavily parasitised (e.p.g. > 1570) were similarly treated.

The stressors of dehydration and cold, pregnancy, the application of insecticide or the administration of anthelmintic showed no evidence of enhancing the toxic effects of copper. However the stressors of starvation, heat, and parasitism did potentiate toxicity and resulted in approximately half of the sheep in each group dying from copper toxicity.

A further series of experiments used 60 sheep, divided into 14 groups; each group being given a different schedule of copper administration which consisted of one of the stressors and/or one of a series of formulations of copper consisting of salts made up in various bases. Blood samples were collected hourly for 16 hours and the rate of change of blood copper concentration was measured. In the sheep that were starved, the rate of change of blood copper concentration increased to 0.141 mg Cu/l/hr for animals starved for 72 hours in comparison with a rate of 0.056 mg Cu/l/hr for animals

given access to food and water. Those sheep that received 50 mg of copper calcium edetate in either of two proprietary formulations; one containing polyvinyl pyrrolidine (PVP) and the other without PVP but the same dose contained in half the volume, showed a mean blood copper concentration rate of increase of 0.017mg Cu/l/hr. An increase in the dose to 100mg Cu increased the rate of uptake of copper to 0.022mg Cu/l/hr, whereas a 50mg dose diluted in an equivalent amount of water showed an increase in the rate of translocation of copper to 0.036mg Cu/l/hr. The four sheep subjected to heat stress or given copper by mouth as copper edetate at a dose rate of 0.33mg/kg showed a blood copper concentration increase to 0.025mg Cu/l/hr, whereas 7gm of oral copper oxide needles administered to three 50kg sheep did not produce any increase in blood copper concentration during the period of study.

Starved sheep also showed changes in their blood concentrations of glucose and albumin. Blood glucose reduced from a mean of 4.7gm/100ml to a mean of 2.4gm/100ml in the nine sheep starved over 72 hours, plasma albumin increased from 1.30gm/100ml to 2.26gm/100ml, and total protein rose by 10.2%.

Deaths following the administration of copper therapeutically have been reported on many occasions. Therefore it was decided to measure the effects of copper therapy on the liver copper storage of sheep which initially had a range of liver copper concentrations. A "copper deficient" farm which regularly reports lambs with enzootic ataxia, and a "copper sufficient" farm with no reported signs of copper deficiency in sheep, were selected. Two hundred sheep on the copper deficient farm and fifty sheep on the copper sufficient farm were treated once annually with 50mg of copper calcium edetate given subcutaneously. This dose was adequate to maintain the liver copper content of all treated sheep on the copper deficient farm above 70 ppm Cu D.M. However in the sheep grazing the copper sufficient farm, liver biopsy samples indicated that copper, apparently surplus to requirements, was stored in the liver resulting in copper concentrations in all sheep in excess of 510 ppm Cu D.M.

Another study measured the uptake of copper by the liver in groups of four sheep of four different breeds common in New Zealand. These breeds were the Border Leicester, N.Z. Romney, Suffolk and Merino. There was no significant difference between the former three breeds, but the Merinos retained less copper in their livers after grazing pasture for 3 months (88ppm vs 164ppm), and also following administration of copper by subcutaneous injection (215ppm vs 330ppm).

The results of this work indicate that certain common stressors met with in everyday sheep management, may enhance copper toxicity. Copper should never be administered to sheep unless the requirement has been confirmed, and at the time of administration particular attention should be paid to avoiding those circumstances that might lead to starvation of the sheep.

ACKNOWLEDGEMENTS

I would like tc thank sincerely my two supervisors, Prof. A.N. Bruere and Dr B.S. Cooper for their guidance, inspiration and ready willingness to discuss this project.

I am grateful to Dr J. Lee of Applied Biochemistry Division of D.S.I.R. for his assistance with analytical methods and in the operation of the inductively-coupled argon plasma emission spectrometer.

The technical assistance received from Miss Karen Armitage and Miss Gaeleen Bell was appreciated.

Mr P.H. Whitehead supplied most of the sheep used in this study and Mr C.K. Barnett spent many hours feeding them and caring for them. The farmers, Mr D.J. Duncan, Hunterville, Mr G. Craine, Feilding, and Mr H.J. Ellison, Tangimoana, were most cooperative and interested in this project. To these people I express my appreciation.

Many people in the Faculty of Veterinary Science gave me willing assistance and I thank them for their cooperation. In particular I thank Mr J.V. Pauli and Mrs E. Davies for their assistance with the clinical pathology, Mrs P. Slack for the preparation of the histological sections, Dr R. D. Jolly for the assistance in the interpretation of the histological sections, and Mrs Janice Tan for the preparation and photography of the electronmicrographs.

This work was financed by Glaxo (N.Z.), Ltd and would not have been possible without their support and encouragement.

Finally, to my wife, Rosemary, and my children Anne, Kirsty, and Stuart , I offer my greatest appreciation for their interest and reassurance throughout the entire period of the project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES