
VOLUME 62 2002

Efficacy of Coprin in deer and sheep: An evaluation and comparative study

T.J. HARRISON, A.R. SYKES AND A.S. FAMILTON

Animal and Veterinary Sciences Group, Lincoln College, Canterbury

ABSTRACT

This study was carried out to examine the efficacy and safety of an injectable copper (Cu) preparation (Coprin; Cu-EDTA, Pittman-Moore Animal Health, NZ) in deer and sheep. Treatments consisted of an injection of Coprinat three dose rates on two occasions five weeks apart. Liver biopsy samples were taken one week before and one and four weeks after each injection and 22 weeks after the second injection.

Approximately 75% of the dose was present in the livers of sheep one week after each injection. However, while over 90% of the dose was present in the livers of deer one week after the first injection, less than 65% of the second injected dose was present after one week. Total plasma Cu was elevated for one week after the second injection only, the increase being proportional to dose rate. Plasma activities of aspartate transaminase, gamma-glutamyl transferase and sorbitol dehydrogenase were transiently elevated after the second injection, though not sufficiently so to indicate a state of toxicity.

The product was found to be effective for rapidly increasing the Cu concentration of the liver in both sheep and deer. There appeared to be no significant ill-effects in either sheep or deer at twice the recommended dose for sheep.

Keywords Copper; Coprin; deer; sheep

INTRODUCTION

An analysis of data from Lincoln Animal Health Laboratory by Familton *et al.* (1985) revealed that, in deer, copper (Cu) appears to be the trace element of major concern to both veterinarian and farmer. Subsequently, the results of a survey of 3500 farmed and 400 feral deer killed in the South Island and processed at the Hokitika, Mossburn or Ashburton deer slaughter premises revealed that liver Cu concentrations of farmed deer were markedly lower than reported normal values for sheep and cattle (Underwood, 1971). Furthermore, feral deer contained at least three times more Cu in their liver than did farmed deer (Harrison and Sykes, pers. comm.), corroborating the findings of Reid *et al.* (1980).

The requirements for supplementation with Cu to deer are not fully understood. Coprin (Pittman-Moore Animal Health, NZ) is a commercially available injectable preparation of Cu-calcium-EDTA registered as a Cu supplement for use in sheep and cattle. This report describes a comparative study of the uptake and retention of Cu by the livers of sheep and deer. Possible toxicity effects were monitored by changes in liver enzyme concentrations in the plasma.

MATERIALS AND METHODS

Twenty four, 3 - 5 year old red deer stags and 24 mixed age Coopworth ewes of bodyweights (mean ± SEM) 144 ± 12.9kg and 55 ± 5.4kg respectively, were used.

Animals from each species were ranked hierarchically according to initial liver Cu concentration and allocated in blocks to one of four treatments. Two subcutaneous injections of Coprin (50mg Cu/ml) were given to the treated animals five weeks apart at the rates given in Table 1. Plastic disposable syringes were loaded with the appropriate doses of Coprin and weighed prior to and after injection to provide an accurate measure of the Cu administered.

Liver biopsy samples were taken by the method of Familton (1985) from all animals one week prior to and one and four weeks after the first injection and one, four and 22 weeks after the second injection. Plasma samples were obtained by jugular venepuncture into heparinised, evacuated tubes immediately prior to injection and then on days 1, 2, 4 and 7 after each of the two injections and thereafter at weekly intervals. Blood samples were kept on ice after collection until centrifugation at 4°C. Plasma was stored at -70°C until analysis.

Both species were allowed to graze the same perennial ryegrass-white clover sward during the experiment. This was supplemented with a silage of similar botanical composition from weeks 19 to 28.

TABLE 1 Mean amount (and SEM) of Cu (mg Cu/kg W) given by subcutaneous injection on two occasions to the treatment groups of each species .

	Control	Low		Med		High	
		Mean	SEM	Mean	SEM	Mean	SEM
Dose 1							
Sheep	0	0.42	0.08	0.74	0.05	0.55	0.22
Deer	0	0.28	0.03	0.58	0.06	1.23	0.17
Dose 2							
Sheep	0	0.67	0.09	1.40	0.14	2.80	0.19
Deer	0	0.54	0.06	1.13	0.09	2.24	0.25

Dried liver samples (approx. 0.2g) were acid digested in a nitric/perchloric (4:1) mixture and plasma samples diluted (1:2) in 6% butanol prior to Cu measurement by atomic absorption spectroscopy. Enzyme activities were determined at 37°C using a micro-centrifugal analyser. Creatine phosphokinase (CPK), g-glutamyl transferase (GGT), alanine transaminase (ALT) and aspartate transaminase (AST) were determined using the diagnostic kits of BGH Biochemical Co., Tokyo. Glutamate dehydrogenase (GDH) was measured using the diagnostic kit of Boehringer-Mannheim (Cat. no. 124 320) and sorbitol dehydrogenase (SDH) by modifications for automation of the manual Boehringer- Mannheim method (Cat no. 125 016) as follows: 200μl of a solution of 15mg NADH, dissolved in 1.5ml 1% $NaHCO_3$, was mixed with 5ml triethanolamine hydrochloride buffer (0.2M, pH 7.4) to form an initial substrate. 20μl plasma sample plus 20μl water was mixed with 125μl of this initial substrate and allowed to incubate at room temperature for at least 45 minutes. After incubation, 20μl of D-fructose (30% w/v) was added and the change in absorbance (340nm) recorded during an 8 minute period.

Total liver Cu content was calculated as the product of liver Cu concentration and liver mass. Dry mass of liver (kg) was estimated from live weight, using the equations:

sheep: $((5.67W) - (0.0285W^2))/1000$ (Langlands *et al.*, 1984)

deer: $((0.011W) + 0.298)0.3$ (Freudenberger *et al.*, 1987).

The animals were weighed on the day of each biopsy sampling after an overnight fast.

RESULTS

The mean body weights of the sheep at the biopsy sampling times one to six were 55, 58, 61, 61, 62 and 69kg (SED 9.6), respectively. The mean bodyweights of the deer at similar times were 144, 149, 154, 154, 156 and 136kg (SED 19.4), respectively. There were no significant differences between treatment groups of either species.

TABLE 2 Liver Cu concentrations (mg/kg DM) of sheep and deer at 0, 2, 5, 7, 10 and 28 weeks after trial commencement. Coprin was administered at one and six weeks.

	Liver Cu concentration (mg/kg DM) Time (weeks)					
	0	2	5	7	10	28
Sheep						
Control	95	91	10	135	136	162
Low	65	139	159	286	246	243
Medium	81	214	222	453	370	354
High	76	328	332	828	680	584
SED	46.0	41.8	35.8	59.4	40.8	64.6
Deer						
Control	53	71	80	84	55	34
Low	51	115	133	223	132	48
Medium	51	187	161	332	205	80
High	48	297	244	543	263	96
SED	15.4	16.4	18.8	37.0	24.2	19.4

Changes in liver Cu concentration are given in Table 2. Copper concentrations in both species were significantly increased one week after the first injection ($P<0.01$), these increases being proportional to the size of dose. Three weeks later, liver Cu concentrations were either maintained or increased (NS) in all sheep treatment groups and in the control and low deer treatment groups, but had decreased in the medium and high deer treatment groups ($P<0.05$).

TABLE 3 The proportion of two successive doses of Cu present in the liver of sheep and deer one week after injection.

	Proportion present (%) Treatment groups			
	Low	Medium	High	
First dose				
Sheep	73.9	74.8	66.7	
Deer	91.8	92.3	81.0	
SED				7.17
Second dose				
Sheep	76.9	66.0	67.5	
Deer	64.9	58.3	52.0	
SED				10.20

Liver Cu concentrations one week after the second injection were significantly elevated ($P<0.01$), these increases again being proportional to the size of dose. Significant decreases were evident in all treated animals of both species four weeks after this injection ($P<0.01$), the rate of decrease being directly related to the concentration one week after injection. The regression equations describing this relationship were:

sheep: $Y = 0.30(\pm 0.03)\,X - 46.6$ ($r^2 = 0.86$)

deer: $Y = 0.54(\pm 0.03)\,X - 26.3$ ($r^2 = 0.92$)

where Y = change in liver Cu concentration (mg/kg DM) and X = post-injection liver Cu concentration (mg/kg DM).

The proportions of the dose retained, calculated as the individual ratio of the increase in liver Cu content (mg) to the dose size (mg), are shown in Table 3.

Total plasma Cu concentrations increased within 24 hours after Cu administration. The increase was small (<0.2mg/litre) after the first injection, but much larger after the second injection, particularly at the high dose rate, when the changes (mg/litre) 24 hours later were 0.10, 0.26 and 0.75 and 0.16, 0.35 and 0.53 for the low, medium and high sheep and deer treatment groups respectively (SED 0.116). These increases had subsided within four to seven days.

Enzyme activities in plasma showed no response to the first injection in either species (Table 4). However, the activities of AST, GGT and SDH were transiently elevated in both species in response to the highest dose at the second injection.

One week after the second dose, four stags of the low treatment group were found to have a small (< 5cm diameter) subcutaneous nodule at the site of the injection. Excision and histopathologic analysis revealed a fibrous and polymorphic infiltration.

DISCUSSION

The efficient uptake of Cu from the first injection by both sheep (72%) and deer (90%) indicated that this preparation of Cu was readily translocated to the liver. Although this efficiency appeared to remain relatively constant over the wide range of doses given to sheep, liver uptake in deer decreased to approximately 60% with the second, higher doses.

This lower efficiency in deer may partly reflect an inability to sequester all of the Cu given as a

TABLE 4 Mean activities (IU/litre) of enzymes aspartate transaminase (AST), gamma-glutamyl transferase (GGT) and sorbitol dehydrogenase (SDH) in the plasma of sheep (S) and deer (D) of the high treatment group at 0, 1, 2, 4, 7 and 14 days after the second injection of Coprin. The mean activities (±SD) of the control animals are given for the 14 day period. PSD is the pseudo standard deviation, calculated as the inter-quartile range divided by 1.35 (Koopmans, 1981).

	Control		Days after injection						PSD
			0	2	5	7	10	14	
AST -S	94	±6.6	105	251	823	395	287	155	24.6
-D	47	±7.3	44	112	351	136	111	53	22.4
GGT -S	52	±6.5	68	85	108	99	69	60	13.3
-D	45	±4.1	44	77	74	66	52	48	13.1
SDH -S	17	±1.5	11	56	429	70	54	42	9.8
-D	7	±0.8	7	30	77	20	13	8	2.5

large dose. However, uptake of the second injection by deer on the low treatment (65%) was less than that on the high treatment of the first injection (81%), even though the dose was smaller. This suggests that the lower uptake by the deer is more a function of liver Cu concentration than size of dose. If this is the case, it may well be that in animals of a lower Cu status than these, efficiencies closer to 100%, similar to those reported by Harvey and Sutherland (1953) and Camargo *et al.* (1962), may be attained.

Although copper concentrations in the livers of treated sheep and deer attained a similar range one week after the second Coprin injection, values remained elevated for a much longer period in sheep than in deer. If the 'critical' liver Cu concentration (at which clinical deficiency is manifested) is similar for deer and sheep (< 25mg/kg DM, Egan 1975), the greater rate of loss by deer suggests that the period of prophylaxis against deficiency in deer would be considerably shorter than in sheep.

In agreement with Mahmoud and Ford (1967) and Ishmael *et al.* (1971), dose rates of less than 2mg Cu/kg W did not appear to induce toxic effects, as measured by liver enzyme activities in the plasma. Even at a dose rate approaching 3mg Cu/kg W, the increases in enzyme activities in the present study were small in comparison to the 100 fold increases reported as a result of liver dysfunction, damage or toxicity (Ford, 1967 ; Ishmael *et al.*, 1971). It may be possible, therefore, to administer twice the dose rate recommended for sheep (i.e. 1mg Cu/kg W) to deer without expecting acute toxicity. However, because of the variability of conditions under which Coprin might be used (eg. Farquharson, 1984), there can be no guarantee of an absolutely safe dose.

The incidence of a local reaction at the injection site was low, both in number and severity. Large abscesses have been produced by Cu-EDTA injections in cattle (G. Judson, personal communication) and in deer (G. van Reenen and P. Holmes, personal communications). Normal procedures of hygiene requirements were followed during the two injections of Coprin. Whether the conditions were completely sterile or the previously reported abscesses were necessarily of microbial rather than chemical origin, is not known. It is perhaps significant that all abscesses were found at low rather than high dose rates.

The data from this study suggest that a dose of 1mg Cu/kg W given to a 150kg stag (i.e., 3ml Coprin) and to a 50kg sheep (i.e., 1ml Coprin) of normal Cu status will produce increases in liver Cu concentrations of 200 and 150mg/kg DM respectively.

REFERENCES

Camargo W.V.; Lee H.J.; Dewey D.W. 1962. The suitability of some copper preparations for parenteral copper therapy in sheep. *Proceedings of the Australian Society of Animal Production* 4:12-17.

Egan A.R. 1975. Diagnosis of trace element deficiencies. *In* Trace elements in soil-plant-animal systems. *Eds.* D.J.D. Nicholas; A.R.Egan Academic Press, New York.

Familton A.S. 1985. A liver biopsy technique for use in red deer. Proceedings of the Deer Branch NZVA, Course 1:60-63.

Familton A.S.; Freudenberger D.O.; Sykes A.R. 1985. Trace elements in deer. Proceedings of the Deer Branch NZVA, Course 1:32-38.

Farquharson B.C. 1984. Some aspects of copper toxicity in sheep grazing New Zealand pastures. PhD Thesis, Massey University.

Ford E.J.H. 1967. Activity of sorbitol dehydrogenase in the serum of sheep and cattle with liver damage. *Journal of comparative pathology* 77:405-411.

Freudenberger D.O.; Familton A.S.; Sykes A.R. 1987. Comparative aspects of copper metabolism in silage fed sheep and deer. *Journal of agricultural science, Cambridge* 108:1-7.

Harvey J.M.; Sutherland A.K. 1953. Parenteral copper therapy in ruminants. *Australian veterinary journal* 29:261-268.

Koopmans L.H. 1981. *In* An Introduction to Contemporary Statistics, Duxbury Press, Wadsworth, Belmont California pp 65-68.

Langlands J.P.; Bowles J.E.; Donald G.E.; Smith A.J. 1984. *Australian journal of agricultural research* 35:701-709.

Underwood E.J. 1971. *In* Trace Elements in Human and Animal Nutrition. Third edition, Academic Press, New York. pp 57-115.