PRELIMINARY REPORT OF THE LIVER:SERUM COPPER RELATIONSHIP IN RED DEER

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INTRODUCTION:

There is little published scientific information about trace elements in deer. The paucity of information has made investigation of trace element deficiencies difficult, particularly in the live deer. Reid et al (1980) indicated that in Otago/Southland farmed deer, liver Cu concentrations in foetuses and neonates (up to 30 days of age) were higher [5589 umol/kg Wet Matter Basis, (WMB)] than in 1 to 6 month-old deer (361 umol/kg) which in turn were higher than in deer older than 6 months (165 umol/kg). Further, these authors showed liver Cu concentrations in mature feral NZ type wapiti were lower than those of feral red deer (327 and 613 umol/kg, respectively). McTaggart et al (1981) reported red deer mean (± SD) liver Cu concentrations of 244 ± 210 umol/kg WMB from the Isle of Rhum (Scotland). Cowie (1976) found a wide range of liver Cu concentrations in deer shot in the north of Scotland (14.3-993.4 umol/kg WMB). None of these authors quoted serum or plasma Cu concentrations or related these to liver Cu concentrations.

Familton et al (1985) reported a poor relationship between liver and serum Cu concentrations in four deer sampled repeatedly during a depletion-repletion study. However, Clark and Hepburn (1986) produced data which conflicted with the conclusion of Familton et al (1985). Clark and Hepburn reported data from 132 paired liver/serum samples, 88 of which were presumably collected from a Deer Slaughter Premises (DSP) while 44 were from clinical cases submitted to the Ruakura Animal Health Laboratory. Results indicated that when serum Cu levels were less than 8 umol/l then liver Cu levels were less than 100 umol/kg. Clark and Hepburn suggested that the "normal range" for serum Cu in deer was 8-22 umol/l.

The liver is the most important storage organ for Cu in the body and therefore analysis of liver samples should give the best indication of the Cu status of the animal. Liver biopsy in deer can be performed and the technique has been described by Familton (1985). However, with valuable animals such as deer the risk associated with liver biopsy may not be acceptable to some owners. It is often not possible to collect liver samples at slaughter within the short time necessary for a diagnosis and correction of Cu deficiencies. It was therefore considered desirable to establish whether analysis of serum would be useful to assist the diagnosis of Cu deficiency in the live deer.

This paper presents data of Cu concentrations of paired liver and serum samples from 426 red deer and establishes the significance of a serum estimate in relation to the liver store of copper within the animal.

MATERIALS AND METHODS:

During the past four years, 426 paired liver/serum samples have been collected as detailed in Table 1. Deer were all 6 months of age or older and the majority (approximately 85%) were stags. Herd G was

in Canterbury and samples were collected at slaughter when the farm was depopulated because of a persistent tuberculosis problem. However, all deer sampled from this herd were clinically normal at the time of slaughter. Samples from Invermay (Otago) and Farm C (South Canterbury) became available when stags were slaughtered for meat studies. Samples for the fourth group were from stags from a number of properties in the lower half of the North Island and were collected at random from a deer slaughter premise (DSP).

Copper in serum and liver from the south Island deer was assayed by the Invermay AHL using a Perkin Elmer 4000 Atomic Absorptiometer after digestion in nitric and perchloric acid. Samples from the NI were analysed by the analytical laboratory of the Veterinary Clinical Sciences Department Massey University using an inductively-coupled argon plasma emission spectrometer after nitric acid digestion.

Liver Cu units are expressed on a wet (fresh) matter basis (WMB, or FMB).

	Month												
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Annual
FARM G													
No. samples			184							44			228
Mean liver Cu*			481.5							190.7			425 4
Range (min			60							23.6			
(max			1598.0							1071.2			
Mean serum Cu**			13.3							10.1			12.7
Range (min			3.9							4.4			
(max			24.8							19.4			
INVERMAY													
No. samples		10	17	13	4	15	0	0	0	0	0	18	77
Mean liver Cu		242.9	296.0	211.8	108.9	137.2						161.1	202.7
Range (min		47.2	31.5	50.4	51.9	40.9						32.9	
(max		756 6	619.5	529.0	179 3	339 8						508.1	
Mean serum Cu		14.8	18 9	22.6	17.3	6 8						9.5	14.4
Range (min		6.1	13.4	13 3	2 0	3 2						6 4	
(max		26 9	24.3	29 8	24.7	9 9						14 9	
FARM C													
No samples									4			4	8
Mean liver Cu									54.3			31 9	43.1
Range (min									47.2			22.0	
(max									59.8			39.3	
Mean serum Cu									3.4			10 4	6 9
Range (min									1.6			9.7	
(max									5.2			13.5	
COMBINED NI FARMS													
No samples	12	19				31	10		9	20		12	113
Mean liver Cu	149 0	229.8				180 0	87 8		81.2	137 8		204.5	154 2
Range (min	14 3	23.8				1.4	41.5		54 3	22.4		37.2	
(max	364.2	736.0				622.5	156.8		121.6	604.4		527.2	
Mean serum Cu	10 2	9.4				11.5	13 4		4.8	11.1		6.7	10.1
Range (min	7.7	1.6				1.4	9.1		1.6	3.9		3.9	
(max	11.2	13.1				20.5	17.3		9.0	21.2		12.4	
COMBINED DATA													
No. samples	12	29	201	13	4	46	10		13	64		34	426
Mean liver Cu	149.0	234.3	465.9	211.8	108.9	166.0	87.8		72.9	174.2		161.2	308 7
Mean serum Cu	10.2	11.3	13.8	22.6	17.3	9.9	13.4		4.4	10.4		8.6	12.2

TABLE 1: Source of specimens and summary of serum and liver copper estimations (umol/l and umol/kg respectively).

RESULTS AND DISCUSSION:

(i) Serum: Liver Cu relationship
Data is presented in Figure 1.

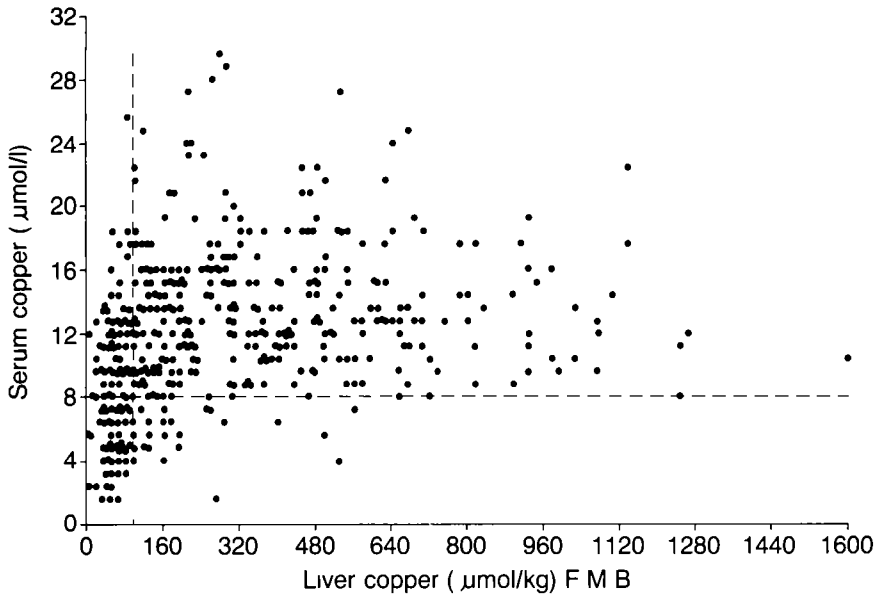


Fig. 1: A graph of Cu concentrations in paired serum and liver samples for all deer in the survey (426). Dotted lines indicate the critical levels of 8 µmol/l and 100 µmol/kg for serum and liver Cu concentrations respectively (see text).

Analysis of this data shows that 47% of deer with liver Cu concentrations < 100 umol/kg had serum Cu concentrations of < 8 mol/kg. This finding is in close agreement with data of Clark and Hepburn (1986) who stated ..."when serum Cu levels are less than 8 umol/l, liver Cu levels are less than 100 umol/kg". The only difference between out data and that of Clark and Hepburn was that 100% of those author's data fell within the stated ranges while we found that a proportion of deer had serum Cu of <8 umol/kg but liver Cu of >100 umol/kg.

Data indicates that when liver Cu concentrations are <100 umol/kg approximately 50% of deer have serum Cu concentrations of >8 umol/kg. It is uncommon to find a serum Cu concentration of <8 umol/l when live Cu concentration is >100 umol/kg. A similar relationship is reported in cattle (Claypool et al 1975).

The liver acts as a store for Cu and when intake is insufficient for metabolic requirements, the animal's homeostatic mechanism draws upon that store to maintain blood Cu concentrations of >8 umol/l. However, when the liver store becomes progressively depleted (<100 umol/kg) an increasing proportion of animals are unable to maintain these "normal" serum Cu concentrations. While the relationship is not absolute, it would appear reasonable from a diagnostic point of view to consider liver Cu concentrations of <100 umol/kg to be "deficient" (see

(iii) below). In cattle, it is accepted that serum Cu concentrations of <7.8 umol/l, corresponding to liver Cu concentrations of <47 umol/kg, represent deficiency (Grace 1983).

In consideration of our data and that of Clark and Hepburn (1986) it becomes evident that the likely reason for the failure of Familton et al (1985) to demonstrate a relationship between serum and liver Cu concentrations in their depletion/repletion studies was that they observed few liver Cu concentrations of <100 umol/kg. The relationship becomes less distinct when only high serum and liver concentrations are considered.

There are probably several reasons for the wide range of serum Cu concentrations when liver Cu concentrations were <100 umol/kg. It is well known that individuals have widely differing Cu metabolism. In addition, it is recognised that stress, especially at the time of sampling, and concurrent disease or disorders can both raise serum Cu concentrations (Underwood, 1981).

(ii) The clinical significance of serum Cu estimations

In order to assist the diagnosis of Cu deficiency/sufficiency in a herd by measurement of serum Cu, at least 10 blood samples should be analysed. If 50% of a herd have liver Cu concentrations of <100 umol/kg the binomial distribution predicts that the probability of finding at least three deer with liver concentrations of <100 umol/kg in a random sample of 10 is approximately 95%. Of these three, 50% will have a serum Cu concentration of <8 umol/kg (i.e 1-2 of the 10 sampled). Thus, the accuracy of a diagnosis is greatly diminished if only a small number of samples are analysed.

Our data suggests that if 50% of serum Cu concentrations are <8 umol/l, then approximately 70% of deer in the herd will have liver Cu concentrations <100 umol/kg. However, this situation represents a severe deficiency, and this is uncommon in practice. It would be unlikely to find 100% of serum Cu concentrations <8 umol/l unless the deficiency was extreme.

There is debate about the use of "mean" Cu concentrations for diagnostic purposes but we suggest that individual results should be assessed in view of the distributions discussed above.

(iii) The relationship between serum and liver Cu concentrations and clinical disease

Enzootic ataxia is a primary syndrome associated with low copper concentrations in deer. The relationship between low Cu and other conditions e.g. bone and joint disorders, ill thrift, has not been firmly established.

In histologically confirmed cases of enzootic ataxia diagnosed in New Zealand (Clark and Hepburn, 1986; Mackintosh et al, 1986) the liver Cu concentrations were all <60 umol/kg. Of the 66 liver samples in the present study with Cu of <60 umol/kg, 50% had serum Cu of <8 umol/l and 66% were <10 umol/l. McTaggart et al (1981) cited his own data and that of Barlow et al (1964) from park deer in the UK with enzootic ataxia. Liver Cu concentrations ranged from 11.2-74 and 33.4-96 umol/kg respectively (after transformation from mg/kg DMB)

Thus, liver Cu concentrations in all reported cases of enzootic ataxia fall below 100 umol/kg.

No serum Cu concentrations were reported from affected deer in studies of McTaggart et al (1981), Barlow et al (1964) or Clark and Hepburn 1986. Mackintosh et al (1986) on the other hand, showed serum Cu concentrations to range from 0.5-2.3 umol/l in wapiti deer with clinical enzootic ataxia. Serum of in-contact red, wapiti and hybrid deer had ranges of 3.8-5.8, 1.0-5.5 and 1.5-2.5 umol/l, respectively. Liver Cu concentrations in in-contact deer (red, hybrid and wapiti) ranged from 20-62 umol/kg.

It would appear that measurement of serum Cu concentrations in both clinically affected deer and in unaffected herd mates provides a useful indicator of herd copper status.

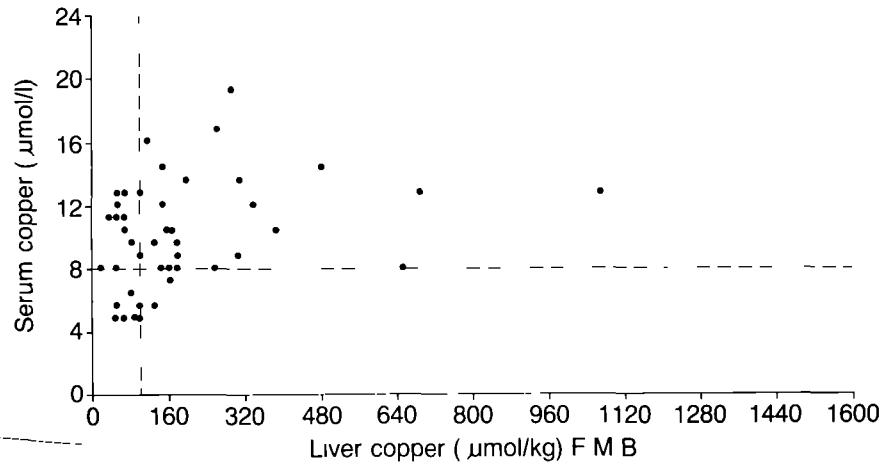
(iv) Effect of season on copper concentrations

Data are presented in Table 1.

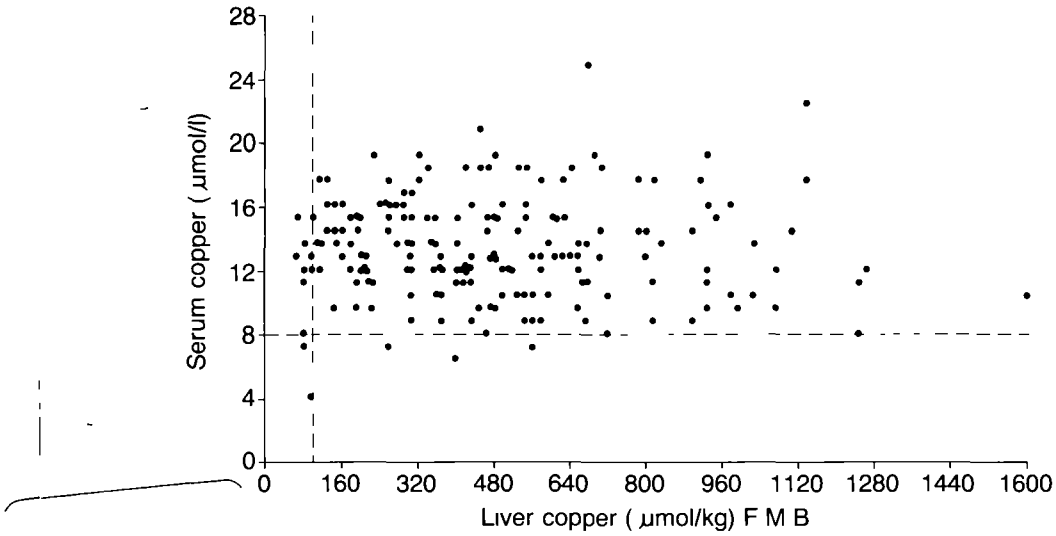
Data indicates that both liver and serum Cu concentrations are lowest during the late winter-early summer period.

It would appear that the uptake of Cu falls in deer during winter as it does in other grazing species. This results in a reduction in liver Cu stores, in many deer, to a point of "deficiency". Grace (1983) reported that soil ingestion and parasite burdens can reduce Cu uptake.

If the Cu deficiency on a farm is severe, the diagnosis may be possible by animal tissue analysis at all seasons. However, in many instances the deficiency will be seasonal. In this case late winter-spring would appear to be the optimum time for sample collection. To highlight this contention, data in Figs 2 and 3 shows the distribution of Cu concentrations from the same farm when deer were sampled in October and March respectively.



graph of Cu concentrations in paired serum and liver samples from 44 deer collected in October from farm G.



graph of Cu concentrations in paired serum and liver samples from 184 deer collected in March from farm G.

(v) Species and breed differences

The data presented here are from red deer. Caution must be exercised in extrapolating this data to other deer species. Wapiti appear more susceptible to copper deficiency manifest by enzootic ataxia than do red deer (Mackintosh et al 1986). Serum and liver Cu concentrations in wapiti were lower than in red deer in that study, suggesting either a higher daily requirement or a poorer uptake of Cu in wapiti. Reid et al (1980) found lower liver Cu concentrations in feral wapiti than in feral red deer (315.6 and 642.1 umol/kg, respectively) from the same environment.

(vi) Effect of Age on Cu concentrations

This study analysed samples only from deer 6 months of age or older. The literature suggests that deer of less than 6 months of age have higher liver Cu concentrations than those of deer older than 6 months (Reid et al 1980). It could be reasoned that serum Cu concentrations would be correspondingly higher in young stock on Cu

deficient properties than in older stock on those properties. There probably would be little difference in serum Cu concentrations between different age groups on copper sufficient properties. It has been noted in at least one instance that serum Cu in weaners (March) ranged from 10.8-17.5 umol/l (mean 14.3) while those in adult hinds at the same time ranged from 2.1-14.0 umol/l (mean 10.0) (Wilson, pers. obs.). Thus, it would appear advisable to avoid sampling weaners when investigating the Cu status of a herd.

High Cu concentrations found in neonate and young deer may be the reason why enzootic ataxia has not been reported in deer of less than 6 months of age.

(vii) Other analyses for investigation of Cu deficiency

A diagnosis of Cu deficiency should ideally be made after analysis of blood, liver, pasture and soil elements, because of the interaction between all of these components in trace element metabolism. For example, high soil pH increases the solubility of molybdenum which in turn contributes with sulphur to the formation of insoluble thiomolybdates in the rumen, rendering Cu less available to the animal. High soil and plant sulphur will also contribute to this phenomenon. Other elements such as iron and manganese will compete with Cu uptake by the animal if they are in high concentrations in pasture (Grace, 1983). Towers et al (1981) found that drenching cattle with zinc resulted in a decrease in blood Cu concentration.

However, Familton et al (1985) suggested that the formation of thiomolybdate complexes occurs to a lesser extent in deer than in sheep. Clearly considerably more research is necessary to establish these interrelationships in deer.

CONCLUSION:

There is good evidence to suggest that liver Cu concentrations of less than 100 umol/kg could indicate deficiency in farmed red deer. Cu concentration will be less than 8 umol/l in 50% of deer with liver Cu concentrations < 100 umol/kg. It would appear that 60 umol/kg represents the "critical" liver Cu concentration, below which enzootic ataxia may occur in some deer. There has not yet been defined a relationship between that liver Cu concentration and other clinical signs of copper deficiency.

Assessment of probabilities suggests that analysis of 10 blood samples should be sufficient to detect a Cu deficiency: e.g. if 3 or more samples are <8 umol/l, it is possible that approximately 70% of deer in the herd will have liver Cu concentrations of less than 100 umol/kg. It is necessary to consider individual sample results rather than mean concentrations to establish this possibility. Seasonal variations indicate that liver and serum copper concentrations are lowest in late winter-spring. Deficiency is more likely to be detected in deer 6 months of age or older. Soil and pasture samples should be analysed in conjunction with blood and liver specimens to complete a full investigation of Cu deficiencies. Repeated samplings may be necessary on some properties, particularly if the deficiency is marginal and correction of Cu deficiencies should be monitored over a period of time.

There remains a considerable void in our knowledge of many other aspects of Cu metabolism in deer including daily requirements, seasonal changes, absorption and excretion, and of the treatment of choice, and the effectiveness and longevity of effect of various forms of supplementation.

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