# A re-evaluation of trace element reference values for farmed deer in New Zealand

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## Abstract

Clinical reports indicate that enzootic ataxia and osteochondrosis occur only when liver copper concentrations are below 60 µmol/kg fresh tissue, and blood copper concentrations are below 3-4 µmol/L Growth responses to copper supplementation have been equivocal in deer when blood copper concentrations were 3-4 µmol/L but were significant when mean blood copper concentration was below 1 µmol/L No antler growth or bodyweight response to copper supplementation were observed when blood ferroxidase activties ranged 10-23 IU/L (serum Cu 6-13µmol/L)and liver coppers ranged 41-98 µmol/kg fresh tissue These data suggest that currently accepted reference values for copper concentrations of 8 µmol/L for blood and 100 µmol/kg fresh tissue for liver, broadly extrapolated from sheep and cattle reference ranges, may be higher than needed for protection against disease and dysfunction in farmed deer.

White muscle disease has been reported in young deer with blood and liver selenium concentrations of 84-140 nmol/L and 240-500 nmol/kg fresh tissue, respectively Recent evidence showed no growth rate response to selenium supplementation in red deer when blood selenium was less than 130 nmol/L, the range in which a growth rate response would be expected in sheep.

Surveys of serum vitamin $B_{12}$ concentrations in deer frequently show levels of less than 185 pmol/L, below which growth rate responses are expected in sheep These concentrations persist without clinical or subclinical effects One vitamin $B_{12}$ supplementation trial showed no growth response in deer with vitamin $B_{12}$ concentrations well below this level.

**Key words**: Red deer, trace elements, reference ranges, copper, selenium, vitamin $B_{12}$

## Introduction

Development of tissue trace element reference values to assess the trace element status of animals is a prolonged process based on cumulative understanding of relationships between biochemical measures and clinical disease, and/or production indices such as growth and reproductive performance After thirty years of deer farming in New Zealand it is timely to review trace element reference ranges for deer

Measurement of blood and liver trace element concentrations is widely practiced by veterinarians in New Zealand. When deer farming started in 1970, no tissue reference values were available, so 'best guess' extrapolation was made from sheep and cattle values, some of which themselves were based on little evidence. Studies of "normal" values were conducted by sampling apparently healthy deer, either at slaughter or on farms (Mackintosh *et al* , 1986, Audigé, 1995). These data, however, do not provide an objective benchmark against which diagnosis of adequacy or inadequacy can be made The principles and processes for establishing tissue reference ranges for trace elements have recently been reviewed (Wilson and Grace, 2000)

There is widespread belief amongst deer farmers that copper deficiency is common, and significant supplementation is undertaken, albeit ineffectively and inappropriately in some circumstances (Wilson and Audigé, 1998) Selenium and vitamin$B_{12}$ supplementation is also undertaken (Audigé, 1995), but there is little information as to the extent and appropriateness of supplementation

To interpret biochemical data it is essential to define "adequate", "marginal" and "deficient" ranges. There is now sufficient evidence from farmed deer that extrapolation of these ranges from other species is not always appropriate This paper re-examines biochemical reference criteria for copper, selenium and vitamin B12 for farmed red deer, adopting the principles for establishing trace element

reference ranges described by Wilson and Grace (2000) It is based on a review of all published material from production response trials, observational studies and relationships between clinical disease and biochemical measurements to date.

## Reference range data for red deer

### 1. Copper

*Normal distribution of data*

Mackintosh *et al* (1986) presented the normal distribution of copper concentrations in paired serum and liver samples from 426 deer and proposed a method for interpretation of data in the four quadrants defined by blood copper concentrations above or below 8 µmol/l and liver copper concentration above or below 100 µmol/kg fresh tissue A similar pattern was observed by Clark and Hepburn (1986) While this data describes the distribution of blood and liver copper concentrations, it cannot provide guidelines for reference values since they were not related to clinical disease or production outcomes Reference values of 8 µmol/l for blood and 100 µmol/kg for liver proposed by Mackintosh *et al* (1986) were extrapolated from cattle reference ranges, in the absence of suitable deer data at the time

*Growth and production response trials*

Table I summarises published data of tissue copper concentrations and growth rate responses in red deer Harrison and Familton (1992) showed a significant growth response in rising 2-year-old deer during spring, when mean copper concentration was initially 5 µmol/L but not during the winter when initial blood copper concentration averaged 12 5 µmol/L

A significant growth response was observed by Ellison (1995) when initial mean blood copper concentration was 4 0 µmol/L and concentrations averaged 0.9-4.0 µmol/L during the trial. This data suggests that a growth rate response may be anticipated when mean blood copper concentrations are 5µmol/L or less However, in the trials by Wilson (1989) and Killorn *et al.* (1991), mean blood copper concentrations were below those levels at times during their trials, yet no significant growth responses were observed Ellison (1995) also demonstrated that between April and June, there was 0.36kg liveweight gain for every 1 µmol/L increase in serum copper and between August and November, there was a liveweight gain of 0 5kg/ µmol/L Seasonal differences probably reflect physiological differences in growth potential

There is anecdotal suggestion that copper deficiency can reduce velvet antler growth. Walker *et al* (1997) were unable to show an antler growth response when liver copper concentrations averaged 98 µmol/kg fresh tissue and blood ferroxidase concentrations ranged from 10 to 23 IU/L (6-13 µmolCu/L) (> 16 IU/L is the adequate reference range for cattle).

Thus, it is difficult from current data to determine the reference value for copper in relation to growth and production in farmed deer What is apparent is that to achieve production responses, blood copper concentrations must be considerably below the currently proposed "adequate" blood copper concentration of 8 µmol/L and liver copper concentration of 100 µmol/kg fresh tissue for liver.

#### *Enzootic ataxia and osteochondrosis*

Table II summarises published literature and veterinary diagnostic laboratory data for confirmed cases of enzootic ataxia and osteochondrosis Liver copper concentrations are available for only 16 deer exhibiting signs of enzootic ataxia. With the exception of one deer with a copper concentration of 86 µmol/kg fresh tissue, all others were below 60µmol/kg fresh tissue as proposed as the 'at risk' range by Mackintosh *et al.* (1986). This data must be interpreted with care, as there was no reporting of copper supplementation of clinically affected animals. The limited amount of blood copper data available shows means of 2.7 µmol/L or less in affected deer and means of 5µmol/L or less in in-contact deer (Table II)

**Table I. Summary of copper supplementation trials in red deer relating growth responses to copper tissue concentrations**

| Author | Age (month) | Mean (range) blood copper (µmol/L) | | Mean liver (range) copper (µmol/kgfresh tissue) | | Response to treatment (kg) |
|---|---|---|---|---|---|---|
| | | *Before trial* | *During trial* | *Before trial* | *During trial* | |
| Lawrence (1987) | 4-12 | | | 360-500 | | 0 (NS) |
| Wilson (1989) | 3-11 | 9 3 (2-19) | 3 1 (0 1-6 7) | 74 (67-80) | | + 3 1 (NS) |
| Killorn *et al* (1991) | 5-14 | 6 5 | 2 3-8 8 | | | +3 4 (NS) |
| Killorn *et al* (1991) | 5-14 | 9 8 | 5 9-12 5 | | | -0 3 (NS) |
| Killorn *et al* (1991) | 5-14 | 11 9 | 5 4-13 7 | | | +1 4 (NS) |
| Killorn *et al* (1991) | 9-11 | 7 5 | 7 9-13 5 | | | +0 4 (NS) |
| Harrison & Famílton (1992) | 14-20 | 12 5 | 5-13 | 130* | 40-210* | 0 (NS) |
| | 20-26 | 5 | 8-12 | 40* | 40-210* | 6 (S) |
| Ellison (1995) | 4-11 | 4.0 | 0 9-4 0 | | | 10 (S) |
| Walker *et al* (1997) | 17-24 | 18 3† | 10 23 | | | +1 9 (NS) |

NS = not significant
S = significant
*Approximations only, extrapolated from graph
†Measured by ferroxidase (IU/L)

**Table II.** **Summary of published literature and veterinary diagnostic laboratory data from New Zealand, 1975-99*, relating confirmed copper deficiency disease and corresponding tissue copper concentrations in red deer.**

| Disease | Year | No. Deer | No. Affected | Age | Liver Cu (µmol)/kg fresh tissue | | Blood Cu (µmol/L) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *Affected deer* | *In-contact deer* | *Affected deer* | *In-contact deer* |
| Enzootic ataxia | 1998 | 58 | 4 | MA | 32, 47, 86 | 17 | | |
| | 1998 | 80 | 5 | MA | 52 | | | |
| | 1997 | NS | NS | NS | 29-60 | | | |
| | 1991 | NS | 3 | 3 yr | 16 | | 2 7 | |
| | 1986 | 100 | 1 | 5 yr | 33 | | | |
| | 1986 | 35 | 8 | 2 yr | 59 | | | |
| | 1986 | 250 | 7 | 18 month | 22 | | | |
| | 1986 | 100 | 3 | 20 month | 17 | | | |
| | 1986 | 56 | 1 | MA | 21 | | | |
| | 1986† | 54 | 5 | MA | | | 0 5-2 3 (mean 1 5) | 1 – 5 (mean 2 5) |
| | 1984 | NS | 3 | NS | 23 | | | |
| | 1982 | NS | 1 | NS | 51 | | | |
| | 1979 | 160 | 5 | 18 month | 45,45 | | | |
| | 1979xx | 150 | 2 | MA, 9 months | | | | |
| OC/BD | 1997 | NS | NS | 1-2 month | 26 | | | 1-5-6 0 (mean 3 3) |
| | 1995 | NS | 8 | 2 months | 23 22 | | 3 | |
| | 1994 | NS | NS | Calves | 32,33 | | | |
| | 1994 | 80 | 6 | NS | 42 | | | |
| | 1994x | 8 herds | summarised | Calves | 0-53 (mean 34) | | | 0 8-5 1(herd means) |
| | 1991 | 160 | 3-4 | Calves | 33 | | | |
| | 1984 | NS | NS | MA | | | | |
| | 1982 | NS | 7 | Fawn | | | Mean 3 1 | |

MA = mixed age (> 1 year) NS = Not stated
OC/BD = osteochondrosis and bone disorders (mainly fractures) Calves/Fawns = usually denotes deer less than 4 months of age
*Source "Surveillance", a quarterly publication of the New Zealand Ministry of Agriculture & Fisheries
† Mackintosh *et al.* (1986) x Thompson *et al* (1994) xx Wilson *et al* (1979)

Data for osteochondrosis and bone disorders (Table II) show liver copper concentrations below 53 µmol/kg fresh tissue and blood copper concentrations of less than 3 µmol/L in affected deer. Mean blood copper concentrations in unaffected herd mates were all below 5.1 µmol/L

### Proposed reference ranges for copper in deer

For liver copper concentrations it is proposed that 60 µmol/kg fresh tissue or less represents the "deficient" range wherein animals are at risk of clinical disease or poor growth rate. Animals in the range 60-100 µmol/kg fresh tissue could be considered "marginal", while those above 100 µmol/kg fresh tissue could be considered "adequate" for immediate needs For blood copper concentrations it would appear that herd means of 5 µmol/L or less represent "deficient" herds, ie deer are at risk of clinical disease or poor growth rate Blood copper concentrations of 5-8 µmol/L could represent a "marginal" range, while concentrations above 8 µmol/lL represent an "adequate" status.

### Selenium

Available data for liver and blood selenium concentrations in deer affected with white muscle disease and those in contact are presented in Table III While this data is limited, liver selenium concentrations in affected deer were 440 nmol/kg fresh tissue or less There are no reports of blood selenium concentrations in white muscle disease-affected deer In one instance, in-contact weaner deer had blood selenium concentrations of 46-74 nmol/L.

Mackintosh *et al* (1989) showed no growth rate response to selenium supplementation when blood GSH-Px concentrations were between 2 7 and 7 9 kU/L. Those authors described a linear relationship between blood GSH-Px and elemental selenium, suggesting that a mean GSH-Px of 2 6 kU/L would equate to a mean blood selenium concentration of a little over 300 nmol/lL

In another study, Grace *et al.* (1999) showed no growth response to selenium supplementation when mean blood selenium concentrations were 227, 97, 141 and 126 nmol/L at intervals during 7 months of observation in rising 1-year-old deer. These authors also demonstrated a significant linear relationship between blood and liver selenium concentrations

The observational study of Audigé *et al.* (1999) indicated that when herd mean GSH-Px was over 3 kU/L, yearling hinds had 11 times higher odds of conceiving early

Thus, while data for selenium reference values is extremely limited, that available suggests that the reference range for selenium deficient sheep and cattle, namely blood and liver selenium < 130 nmol/l and <250nmol/kg fresh tissue, respectively, which is currently used for deer, may be too high More research work is required to establish selenium reference ranges for deer.

### Vitamin $B_{12}$

### Blood and liver vitamin $B_{12}$ concentrations reflect the Co intakes of deer

No weight gain response was observed in two trials with young growing red deer with serum vitamin $B_{12}$ concentrations less than 185 pmol/L, the concentrations at which growth rate responses would be expected in lambs (Clark *et al* 1986) Routine monitoring of blood frequently demonstrates serum vitamin $B_{12}$ concentrations well below 185 pmol/L in clinically normal and rapidly growing deer (Audigé, 1995, Beatson *et al.*, 1999). The observations of Audigé *et al* (1999) indicated serum vitamin $B_{12}$ concentration was positively associated with the probability of yearling hinds conceiving. When analysed as a categorical variable above a threshold herd mean of 185 pmol/L, the relationship was not significant, again suggesting this is not the appropriate reference concentration to assess vitamin $B_{12}$ deficiency in deer.

Beatson *et al.* (1999) demonstrated a non-linear relationship between liver and blood vitamin $B_{12}$ concentrations similar to that observed for serum and liver copper concentrations, confirming serum vitamin $B_{12}$ is not a useful indicator of liver vitamin $B_{12}$ concentration Thus, while data is limited, that available suggests that the reference range for vitamin $B_{12}$ deficiency in deer is below that currently used for cobalt or vitamin $B_{12}$ in sheep, namely serum and liver vitamin $B_{12}$ <336 pmol/L and < 280nmol/kg fresh tissue, respectively More observations are required to validate this conclusion.

**Table III.** **Summary of veterinary diagnostic laboratory data from New Zealand, 1975-99*, relating confirmed white muscle disease and corresponding tissue selenium concentrations in red deer**

| Year | No. Deer | No. Affected | Age | Liver Se (nmol/kg fresh tissue | | Blood (nmol/L) | |
|---|---|---|---|---|---|---|---|
| | | | | *Affected* | *In-contact* | *Affected* | *In-contact* |
| 1977 | NS | NS | 3 month | 73, 140 | | | |
| 1996 | NS | 6 | Fawn+ | 270,260 | | | |
| 1996 | 300 | 5 | Weaners+ | | | | 46-74 |
| 1995 | 60 | 40 | 1 month | 430,440 | | | |

*Source "Surveillance", a quarterly publication of the New Zealand Ministry of Agriculture & Fisheries

NS = Not Stated

+Fawn, weaner = Usually denotes deer less than 4 months

## Conclusions

The need for species-specific trace element reference values is supported by data and observations discussed in this paper The ultimate reference range is one which accurately predicts the probability of a production response of a specified magnitude under any given set of circumstances (Clark *et al* , (1985) These circumstances should relate to specific combinations of breed, age, sex, season, dietary trace element content, soil type, climate and management practices. It is notable that limited information is yet available for most trace elements of deer despite their being farmed for more than 20 years demonstrating the difficulty in establishing robust reference ranges for newly adapted wildlife species While the tissue trace element data support a suggested criteria for "deficient", "marginal" and "adequate" copper status of deer in terms of blood and liver copper concentrations, it is insufficient to categorise the deer in terms of their selenium or vitamin $B_{12}$ status Indications for both the latter trace elements suggest "marginal" and "deficient" ranges may be below those currently used for sheep. Thus, it will be some time yet before the proposition of Suttle (1986) that veterinary intervention should try to prevent animals from entering the marginal status, which precedes the "deficient" state, can be achieved based on biochemical criteria for most trace elements for deer, and most other newly farmed wildlife species

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