A REVIEW OF COPPER AND SELENIUM REFERENCE RANGES IN CATTLE AND SHEEP

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The definitive test to determine if a group of animals require supplementation with a particular mineral is whether they respond in some measurable way to the addition of the mineral relative to a group of controls. The practical difficulties in doing this makes such a test impractical as a routine however. Also, if there is no response it gives no information on the likelihood of a response some time in the future. Testing the actual mineral level in the animal and relating the levels to some predetermined reference range has become the diagnostic test of choice because it is more practical and generally provides information on the degree of sufficiency when levels are in the non responsive range. For this technique to be useful there must be a relationship between the level of mineral in the body and the likelihood of response and the reference ranges must be based on sound scientific principles.

This paper discusses the basis of the reference ranges used by the Animal Health Laboratory Network of MAF Quality Management in New Zealand cattle and sheep for the minerals selenium and copper.

SELENIUM REFERENCE RANGES - CATTLE

There are numerous selenium responsive conditions described in cattle but not all have been confirmed in New Zealand. These are:

Illthrift ± diarrhoea*
Milk production responses*
Fertility responses*
Nutritional muscular degeneration*
Retained foetal membranes
Susceptibility to infection especially mastitis (somatic cell counts)
Birth of premature, weak or dead calves

*Reported in New Zealand.

New Zealand Data

Growth responses in young cattle to supplementation

Blood selenium
Since selenium was discovered to be an essential trace element in 1957, many Se growth response trials have been carried out in New Zealand. Fraser and Wright[1] brought together the results of all the selenium supplementation trials known to have been performed in New Zealand. Data was obtained from 96 trials but in only 39 of these was blood selenium measured. The trials were located from Northland to Otago. Most of the

calves used in the trials were spring born and most of the trials started before the calves were 12 months of age. Both dairy and beef breeds were represented and anthelmintic treatment was used in most.

The data comparing liveweight responses to selenium with blood selenium levels is presented in figure 1. Liveweight gain response is taken as the mean daily liveweight change of Se-treated cattle minus the mean daily liveweight change of controls for a period of up to 3 months. Blood selenium had to be measured within this 3 month period. Twenty two of the 39 trials where blood selenium was measured qualified. The graph indicates that liveweight gain responses to Se tend to increase with lower blood Se levels but responses were highly variable especially at low blood Se levels. When the data was examined for factors which may be contributing to the large variation e.g. breed, level of nutrition, time of year, there was no influence apparent for breed or level of nutrition, but there did seem to be a seasonal variation with a tendency for greater responses in autumn and winter, than in spring and summer. The peak response seemed to occur in May. This is in broad agreement with the clinical observations of Hartley and Grant[2] who found that clinically evident selenium responsive illthrift "largely occurred in the autumn and winter months".

The relationship between whole blood selenium and whole blood glutathione peroxidase has been well established. They are well correlated when cattle have been on a steady state selenium intake over the previous 4-6 months[3]. The reference ranges used by the Ministry of Agriculture and Fisheries for whole blood glutathione peroxidase are derived from the regression equation with whole blood selenium as is the reference range for serum selenium. It must be remembered however that these relationships change when selenium is supplemented[4 (see later)].

Liver

The only work I am aware of where liver selenium levels were measured in calves undergoing a supplementation weight gain response trial was Vickers et al[5]. They found selenium supplemented nine month calves grew significantly faster than controls over a two month period. A liver sample from one calf at the beginning of the trial contained 330 nmol/kg of selenium. Fraser[6] examined the relationship between whole blood and liver selenium from samples taken from the same animal. He found that these two parameters were moderately well correlated and that a blood selenium of 130nmol/l and 250nmol/l was equivalent to a liver concentration of approximately 380 and 650nmol/kg respectively.

Conclusion

The selenium reference ranges currently used for growing cattle by MAF are based on the above trial results and are as follows:

Selenium reference ranges related to growth of young cattle

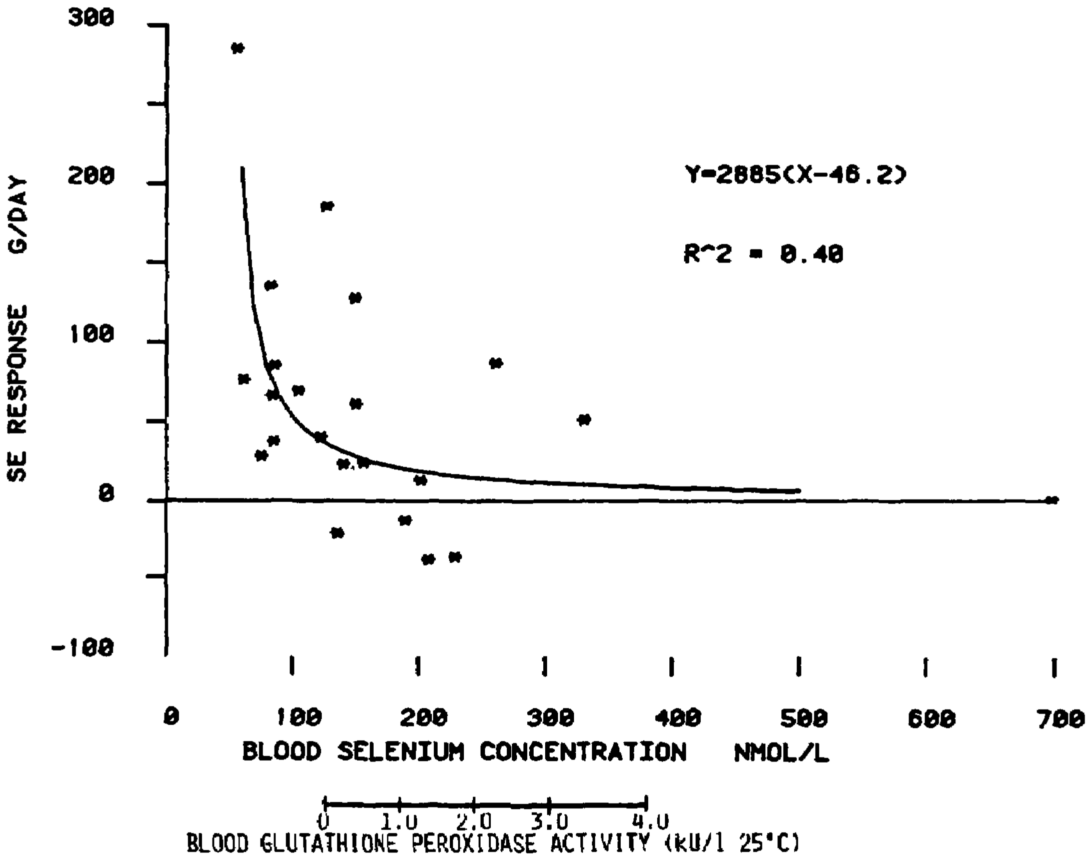
	WHOLE BLOOD (nmol/l)	SERUM (nmol/l)	GPX (kU/l25°C)	LIVER (nmol/kg)
Responsive	<130	<52	<0.5	<380
Marginal	130-250	52-100	0.5-2.0	380-650
Adequate	>250	>100	>2.0	>650

The important points to note are:

1. **Economically significant growth rate responses to supplementary selenium are unlikely at blood selenium levels above 250nmol/l and glutathione peroxidase levels above 2.0kU/l at 25°C. At mean blood selenium levels of <130nmol/l or blood glutathione peroxidase levels <0.5kU/l at 25°C growth responses to supplementation can be expected but the magnitude of response is highly variable being the greatest during the autumn and winter.**

2. ***The liver and serum reference ranges are still provisional as more comparisons need to be made with whole blood selenium.***

Figure 1: Liveweight response against blood selenium level.



Nutritional muscular dystrophy in calves

Nutritional muscular dystrophy in calves appears to be very uncommon in New Zealand. Hartley and Grant found isolated cases on just six pumice properties and one sand property in the North Island and no cases in the South Island[2]. Affected calves were from 1 to 4 months of age. No congenital myocardial cases were seen. The mean selenium concentrations in the liver of affected calves was 445nmol/kg[7]. This level is in the marginal range for selenium responsive illthrift.

Milk production responses in lactating dairy cows

There have been three publications (involving nineteen herds) that have described the effect of selenium supplementation on milk production and related this to blood selenium levels. In all cases milk fat production was measured in both supplemented and control animals over the season. Seven of the herds were on Waikato Peatland[8], twelve were on alluvial pumice and peat soils of the Rangitaiki Plains9 and three herds were on a marginal selenium soil type in Taranaki[10].

A statistically significant increase in milk fat production of 7.4% was obtained on one of the seven Waikato peat farms. This farm had the lowest seasonal average blood selenium level of 80nmol/l. For the other six herds where average blood selenium levels for the season ranged from 110-200 nmol/l the amount of milk fat response to selenium supplementation averaged -2.1%

Figure 2 outlines the results of the trials on the Rangitaiki plains and in Taranaki. The milk fat responses to selenium are plotted against blood selenium levels of control animals. In these trials the average response of milk fat was 3.7% and this occurred at blood selenium levels between 50-150 nmol/l. There was also evidence that the magnitude of response increased with decreasing blood selenium levels.

In addition to these trials there are a number that have not been published. There was no significant response to selenium in three Northland herds with mean blood selenium levels of control animals of 90, 127 and 140nmol/l[11,12].

In seven Canterbury herds, bimonthly injections of 40mg of selenium had no significant effect on milk production as measured by the production index. Control animal blood selenium levels ranged from 97-250 nmol/l[13]. No significant response was also found on two Otago farms with mean blood selenium levels of 133 and 164nmol/l[14].

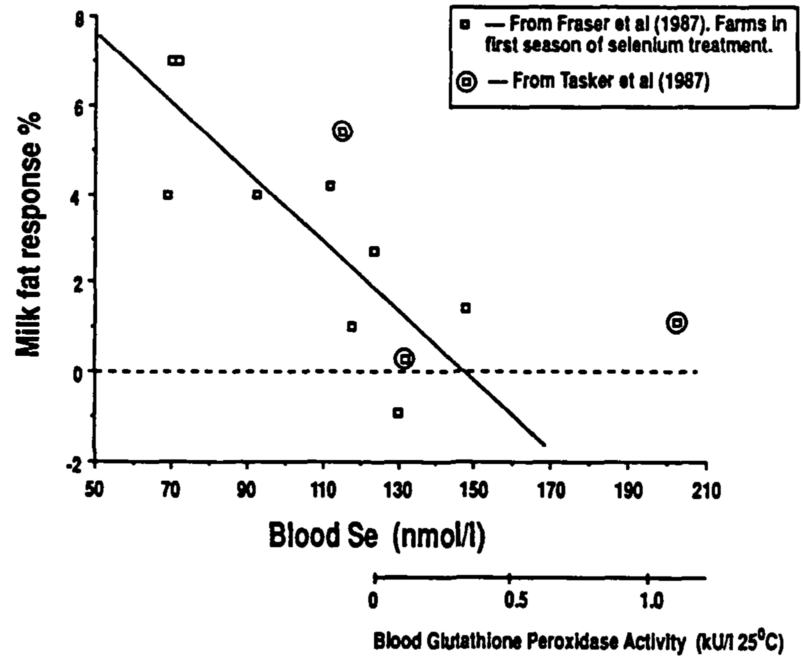
Selenium supplementation with slow release rumenal boluses or depot barium selenate injections did not significantly increase milk production in dairy cows on four Waikato[15,16] and one Manawatu farm[17]. The mean blood selenium level of control cows ranged from 240-545nmol/l.

In total the author is aware of 39 farms where selenium response trials have been conducted in lactating dairy cows. Blood selenium levels of control animals have ranged from 60-545nmol/l which is equivalent to GPX levels from undetectable to 6kU/l 25°C. Statistically significant milk production responses have been detected on 6 of these farms with mean blood selenium levels of control animals ranging from 70-161nmol/l.

Conclusion

The data suggests that economically significant milk production increases to selenium supplementation are unlikely at mean blood selenium levels greater than 250nmol/l and GPX levels greater than 2kU/l 25°C. As with growth rate, responses are variable at blood selenium levels below these values but there is a tendency for the magnitude of response to increase with decreasing blood levels.

Figure 2: Milk fat response to selenium versus blood selenium concentration.



The effect of selenium on dairy cow fertility

The number of trials examining the effect of selenium on fertility in cattle is quite limited and clearly more work is needed to determine the conditions under which fertility responses to selenium supplementation may occur in cattle.

In the Rangitaiki work mentioned above there was no effect of selenium supplementation on the intercalving interval. However this is probably not a very sensitive indicator of cow fertility as it can be affected by other factors such as culling, cow deaths and induction of parturition. In the Taranaki trials Tasker et al did find that selenium supplementation improved both submission and conception rates on two farms with average blood selenium levels of 115 and 130 nmol/l but not on a third farm where the average blood selenium level of the control cattle was 200 nmol/l.

A significant reduction in calving to conception interval was found in a Te Awamutu dairy herd with mean blood selenium levels of control animals of 545nmol/l[15]. There was no such effect in a second herd where blood selenium levels of control animals averaged 354nmol/l.

Scales[18] found that selenium injected to beef cows before mating on farms in the Oamaru district of North Otago had no effect on the proportion of barrenness.

Other trials in South Island dairy cattle have failed to demonstrate a beneficial effect of selenium supplementation on intercalving interval (seven herds) or on days from calving to conception (two herds)[13,14]. Whole blood selenium levels in control animals ranged from 97-271nmol/l.

A further two trials in dairy cows, still in progress, have so far failed to demonstrate a significant effect of selenium on submission rate, conception rate or calving to conception interval. Mean blood selenium levels of control animals in these herds ranged from 240-450nmol/l[16,17].

Conclusion

There is insufficient data to establish a reference range for fertility responses to selenium in cattle but, with one exception, the evidence we do have suggests fertility responses are a possibility only at quite low blood selenium levels, e.g. similar to the milk production response range.

Retained foetal membranes
Increased susceptibility to infection
Birth of stillborn or weak calves

As yet no controlled studies have definitively proven these conditions are selenium responsive in NZ and hence no reference range is available. The effect of selenium supplementation on the number of retained foetal membranes and number of assisted and dead calves was examined by MacKenzie in the seven Canterbury herds mentioned above[13]. The number of cases was so low that there was insufficient data for analysis. Stone[19] found that heifers given an intraruminal bolus that released 3mg Se/day had lower mean somatic cell counts than heifers given 0.8mg of selenium/day by the water system. However numbers of animals were small and the result was not statistically assessed. There are other reports that supplementary selenium reduces number of retained placentae, reduces the number of empty cows, reduces the number of mastitis cases and lowers somatic cell counts. These reports are based on surveys in the Te Awamutu area and the results of supplementing all cows within a herd and observing performance the following season[20]. These findings, although useful, need confirmation using a controlled trial design.

There are claims based partly on overseas data and partly on local uncontrolled data that selenium responses occur at much higher levels than is currently suggested by the MAF reference ranges[21]. It is suggested that blood glutathione peroxidase levels should be >20kU/l at 25°C (whole blood selenium >1460nmol/l) in order that selenium deficiency is not contributing to the number of cases of retained foetal membranes and clinical mastitis and to lowered fertility. In most of the trials quoted above the GPX levels of supplemented animals did not exceed 10kU/l 25°C. However in five of the herds[15,16,17] supplemented cows had GPX levels substantially above 10kU/l 25°C. In four of these herds supplementation to date has had no beneficial effect on submission rate, conception rate, number of days open, milk production, somatic cell count and number of cases of mastitis or retained foetal membranes. In one herd there was a significant reduction in calving to conception interval but no effect on any other of the above parameters.

Conclusion

There is little evidence from trials to date that herds will respond to selenium if their blood glutathione peroxidase levels are greater than the current MAF recommendation of 2kU/l 25°C. It seems justified however to aim for levels above this so that there is some reserve to overcome any shortfall period. The significance of the one herd that did respond at levels considerably greater than this should become clearer as the results from other trials currently being done are published.

Overseas data

The classical syndromes of selenium deficiency like nutritional muscular dystrophy have been well described overseas. In addition there are many reports that selenium ± vitamin E has a role in the prevalence of retained foetal membranes, resistance to and recovery from infections, prevalence of cystic ovaries, cow fertility, heinz-body anaemia and perinatal calf deaths. The relationship, however, between these selenium responsive conditions and the level of selenium in blood appears to be quite poor (see Table 1). One of the reasons for this is the highly variable design used in these studies especially differences in supplementation programmes and basal diets. However there is sufficient evidence in the literature to conclude that there are factors other than the animals blood selenium level that influences whether a response to supplementation will occur. These factors are not yet well defined but their definition probably holds the key to why New Zealand cattle can thrive at blood levels incompatible with life in Northern Hemisphere countries. Some of these factors may be:

1. The vitamin E status of the animals

It is well established that vitamin E and selenium are both important for the protection of cells from oxidant damage and that high levels of one may provide some protection against low levels of the other. In some of the trials in Table 1 the greatest responses occurred when both selenium and vitamin E were supplemented together. The vitamin E status of New Zealand cattle is generally very adequate and levels in the overseas "deficient range" are rare in pastoral grazing situations[22]. This is a likely reason why nutritional muscular dystrophy is rare in New Zealand cattle but common in Northern Hemisphere cattle with blood selenium levels considered very adequate in New Zealand.

2. Degree of microorganism challenge

Selenium has an important role in protecting animals against invasion by microorganisms. In selenium deficiency, neutrophils are less able to kill phagocytosed bacteria and yeasts and antibody responses are reduced. It is therefore possible that selenium levels do not need to be as high under situations where the risk of infectious disease is low. This potentially is another reason for the difference in selenium levels recommended between N.Z. and Northern Hemisphere countries. Microorganism challenge is likely to be much greater when cows are closely confined during long cold and damp Northern Hemisphere winters.

3. Prevalence of disease

It is interesting to note that the prevalence of some of the selenium responsive conditions described overseas is high on New Zealand standards and that supplementation does not necessarily reduce the prevalence to our level. Retained foetal membranes is a good example. In the herds in Table 1 that responded to selenium ± vitamin E the overall prevalence of RFM was 25% in unsupplemented animals and 8% in supplementeds.

Conclusion

There are obviously factors unique to overseas countries which makes it dangerous to extrapolate their data to New Zealand conditions without first validating it. To date most of the trials setup to do this have shown no benefit in maintaining selenium levels at recommended overseas levels.

Table 1: Selenium responsive syndromes reported overseas in relation to blood selenium and glutathione peroxidase and serum vitamin E levels of unsupplemented cattle.

A. Herds that responded to selenium supplementation

SYNDROME	BLOOD Se* (nmol/l)	BLOOD GPX+ (kU/l 25°C	SERUM VIT E (µmol/l)	REF
Retained placentae	790	10	N.M.	23
	952#	12	3.9#	24
	1587	22	N.M.	25
	1870	26	N.M.	25
Reduced fertility	400-1100	4-15	N.M.	26
	2700	39	N.M.	27
Reduced resistance to infection	406#	4	N.M.	28
	419	4.5	14.6	29
	470	5	13.9	30
	940	12	11	31
	952	12	3.9	24

B. Herds that did not respond to selenium supplementation

SYNDROME	BLOOD Se* (nmol/l)	BLOOD GPX+ (kU/l 25°C	SERUM VIT E (µmol/l)	REF
Retained placentae	1000	13	4.6	32
	1050	14	N.M.	33
	1207	16	N.M.	25
	2225	32	N.M.	34
	2445	35	N.M.	25
	2700	39	N.M.	35
Reduced fertility	1000	13	4.6	32
	1100	15	N.M.	36
	2225	32	N.M.	34
Reduced resistance to infection	444	5	14	37
	1050	14	N.M.	33

* Serum converted to whole blood by multiplying by 2.5
+ Derived from whole blood Se using the regression on page
# Supplementary vitamin E also required for maximum effect.
N.M. Not measured.

Relationship between selenium in whole blood, in serum and whole blood glutathione peroxidase

Under steady state selenium intakes these parameters are well correlated. The regression equation between glutathione peroxidase and whole blood selenium has been determined by the Animal Health Laboratory Network. The reference range for glutathione peroxidase has been calculated from this.

It must be stressed that this reference range for glutathione peroxidase only applies to samples analysed by the Animal Health Laboratory Network as they all use the same method. There are many different methods of measuring glutathione peroxidase and each will produce different numerical results. This is not such a problem with selenium as one is measuring the mineral, not an enzyme rate reaction.

When animals have been supplemented with selenium in the previous 4-6 months the relationship between selenium in whole blood, in serum and whole blood glutathione peroxidase changes[3,4]. Whole blood and serum selenium levels rise to peak values within hours of supplementation due to free selenium in plasma/serum. In contrast whole blood glutathione peroxidase activity takes 3 months to reach peak levels as the selenium has first to be incorporated into red cells which occurs only during haematopoiesis. The decay in serum selenium post supplementation is rapid as it is quickly redistributed around the body and excreted. Levels in calves were similar to those in control animals two months after being given 0.1 mgSe/kg bwt intramuscularly[4]. In contrast the decay in red cell glutathione peroxidase is determined by the red cell life span (135-162 days in cattle) so that comparable glutathione peroxidase levels between treated and control calves were not reached until six months post injection. The decay in blood selenium is intermediate between that seen in serum and glutathione peroxidase as it is measuring free selenium in plasma and selenium fixed in the red cell (in the form of glutathione peroxidase).

SELENIUM REFERENCE RANGES FOR SHEEP

Several selenium responsive conditions have been described in sheep and all have been reported in New Zealand.

Selenium responsive unthriftiness in lambs
Selenium responsive condition loss ± diarrhoea in adult sheep
Nutritional muscular dystrophy in lambs - congenital and delayed
Nutritional muscular dystrophy in hoggets
Selenium responsive infertility in ewes.

Selenium responsive unthriftiness in lambs

In New Zealand there have been many trials, mainly in the 1950s and 1960s comparing the growth rate response to selenium supplementation with selenium levels in pasture, blood and liver. As a result of these trials the following reference ranges have been published with the number of published trials at each level also included.

	Blood Se (nmol/l)	No. published trials	Ref
Marked response	<63.5	8	38
Mild-mod response	63.5-127	10	38,39,40
Marginal	128-250	5	38,39,41
Adequate	>250	5	38,39,40

Lambs with blood selenium levels in the markedly responsive range were 4-7kg lighter than selenium supplemented animals at 20-28 weeks of trial and there were often many deaths. In contrast lambs that were mild-moderately responsive were 1-3kg lighter than supplementeds and there were few deaths. There were growth responses of 2-3kg at 18-26 weeks in two of the four trials where blood selenium levels of control lambs were in the marginal range. No growth responses occurred in the other two trials. This difference was possibly because the control lambs in the responsive trial had selenium levels close to the responsive range whereas the other two groups were close to the adequate range.

	Liver Se (nmol/kg)	No. published trials	Ref
Responsive	<250	3	38,40
Marginal	250-450	2	38,39
Adequate	>450	3	38,40

The average growth response to selenium in the trials where the mean liver selenium levels were in the responsive range was 4.5kg at 20 weeks. This compared with 2kg over a similar period for the marginal range. No growth responses were reported when liver selenium levels averaged 940, 2705 and 2030 nmol/kg. However a significant growth response to a selenium supplement was reported in a group of lambs with liver selenium levels in control animals of 1270nmol/l[42].

Selenium responsive condition loss ± diarrhoea in adult sheep

Selenium responsive unthriftiness is also reported in adult sheep[38,43,44,45]. In one trial where selenium supplementation prevented the loss of 5kg of body weight from one month before joining to one month prelambing mean blood selenium levels of unsupplemented ewes at joining was 115nmol/l and one month prelambing was 125nmol/l[38]. This selenium responsive condition has not been extensively studied but it appears to be less common than selenium responsive unthriftiness in lambs and hoggets[45]. Therefore the selenium reference range derived for young sheep should more than cover this.

Selenium responsive infertility in ewes

This results from embryonic loss at 3-4 weeks of gestation[46]. Lambs born from selenium sufficient ewes may also be heavier and ewes milk production better than selenium deficient ewes[45]. Hartley performed many trials to measure the effect of selenium on ewe fertility but blood and tissue selenium levels were not reported[46]. There are just three published trials of selenium responsive infertility where blood selenium levels were measured. In two trials the barren ewe percent of unsupplemented ewes was 41% compared with 9% for the supplemented animals. The blood selenium concentration of undosed animals at joining and one month prior to lambing ranged from 102-125 nmol/l[38]. In the third trial where ewes were slaughtered from 10-36 days in order to study the foetal death further, all unsupplemented ewes slaughtered after 30 days contained dead foetuses whereas all foetuses in the selenium dosed flockmates were alive. Unsupplemented ewes had blood selenium levels below 127nmol/l. In a subsequent year when undosed ewes had blood selenium levels between 150 and 216 nmol/l at slaughter no foetal deaths were reported[38]. The results of these trials suggest that the critical level for foetal survival is less than 150 nmol/l.

Nutritional muscular dystrophy

Blood and liver selenium concentrations reported in New Zealand[7] cases of white muscle disease are as follows:

	Sample	No.of samples	No.of properties	Mean Se nmol/kg(l)	Range
Congenital WMD	Liver	8	2	317	216-597
Delayed WMD	Liver	27	12	609	279-1244
	Blood	30	12	203	76-419

Levels of selenium in liver and blood are not as low on average as levels seen in selenium responsive illthrift. This may be because other factors, especially the balance between the intake of vitamin E and polyunsaturated fats, contribute to the development of nutritional myopathy whereas these do not appear to be factors in selenium responsive illthrift[2,47]. Low liver vitamin E levels are seen on occasions in New Zealand sheep, especially in some cases of nutritional myopathy[22].

Conclusion

1. The selenium responsive disease most closely studied in sheep is lamb unthriftiness and there is a close relationship between the blood selenium level and magnitude of growth response. Therefore the published reference range can be used with some confidence. It is probably preferable that blood be sampled rather than liver as more trials have been done using the former.

2. The reference ranges for the other selenium responsive diseases are not as well established but the data available suggests that if blood levels are in the adequate range to prevent illthrift in lambs then infertility and condition loss in ewes should not be present.

3. Both blood and liver selenium levels need to be higher to prevent nutritional muscular dystrophy. Liver selenium may need to be greater than 1300nmol/kg and blood selenium greater than 500nmol/l in lambs to prevent this disease. However, such a reference range is complicated by the fact that vitamin E levels are also important in this disease.

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COPPER REFERENCE RANGES - CATTLE

The most commonly described manifestations of copper deficiency in cattle are[1]:

- poor growth
- lowered milk production
- diarrhoea (mainly if molybdenum intakes are high)
- achromotrichia
- bone fragility
- post parturient haemoglobinuria (mainly Northland)
- reduced fertility

Growth responses in young cattle to supplementation

Growth response trials from all over the world have been reviewed to obtain data to estimate the live weight responses at varying serum and liver copper concentrations[2]. The growth rate difference in kg/day between supplemented and unsupplemented cattle were plotted against mean serum or liver copper concentrations of control animals over that period. This resulted in 32 points on the serum graph and 16 points on the liver graph. The lines of best fit were then estimated statistically and the figures below derived:

Mean serum Cu (µmol/l) (6-10samples)	Growth response (kg/d)
<1.5	0.33
1.5-5.5	0.18
5.6-7.9	0.08
≥8.0	Nil

Mean liver Cu (µmol/kg) (≥3 samples)	Growth response (kg/d)
<45	0.23
45-95	0.05
>95	Nil

Where whole blood or plasma copper concentrations were reported they were converted to serum copper based on regression equations derived from Ruakura Animal Health Laboratory data.

There is quite strong agreement in the literature that young cattle with mean serum copper levels greater than 8.0µmol/l and liver copper concentrations greater than 95µmol/kg are unlikely to grow better following supplementation. The probability of a growth response and its magnitude increase with:

- decreasing serum or liver levels
- with younger cattle
- with the duration of hypocupraemia[2,3,4].

Another important factor is the mechanism by which copper depletion has occurred. Experimental studies suggest that growth retardation in cattle may only occur if copper deficiency is a consequence of high molybdenum intake as there was no effect on growth when calves were depleted of copper to the same level by a high intake of iron[5,6].

Milk production responses to supplementation

There are only a few published controlled trials detailing the response of adult cattle to copper supplementation.

Cunningham[7] found that copper supplementation of dairy cows grazing peat prevented chronic diarrhoea, poor fertility and low milk yields. Unsupplemented cows had mean serum copper concentrations of 6.3µmol/l and mean liver copper levels of 52µmol/kg. The level of production response was not documented.

In contrast there was no significant milk production response to copper supplementation over 2 seasons in 8 Northern Ireland herds where control animals had mean liver copper concentrations of 41µmol/l and serum levels of 6.3µmol/l. Overall however the response was positive[8].

Copper supplementation increased milk production in hypocupraemic Northland cows but this response was attributed to the prevention of post parturient haemoglobinuria. In most herds where PPH is diagnosed mean serum copper levels are less than 8µmol/l[9,10,11] although a few individual cases have occurred at levels above this and up to 14µmol/l[12].

The Animal Health Centre at Morrinsville and Te Aroha performed controlled trials in five herds over two seasons[13]. All herds were initially copper depleted with mean serum levels ranging from 3.1-6.7µmol/l and liver copper levels between 17-45µmol/kg. In the first season 3 of the 5 herds had a positive production response to copper which reached statistical significance for protein in one herd. The following season there was a positive effect of copper supplementation on the production index in 3 herds and this was statistically significant in two. In the 3 responsive herds the mean liver copper concentration of control cows was 39µmol/l and the mean serum copper concentration was 8.2µmol/l. This compared with 91µmol/l for liver copper and 8.5µmol/l for serum copper in those herds/years where there was no significant response.

Fertility responses to supplementation

The field evidence that copper deficiency causes infertility is conflicting. Cunningham[7] demonstrated that copper supplementation improved fertility of cattle grazing peat and Hunter[14] in New South Wales found that copper injections at calving improved 90-day non-return rate in five herds with mean serum copper levels between 10-11µmol/l. Others however have been unable to demonstrate a significant effect of copper on fertility with mean serum copper levels as low as 4.7µmol/l[15-19]. In a series of experimental studies Phillippo et al. [20,21,22] found that fertility of copper deficient cows may be impaired only if their low copper status is produced by high intakes of molybdenum. They found that the presence of molybdenum, rather than low copper status was responsible for the delays in puberty, reduction in conception rates and disruption of oestrus activity in cattle. Molybdenum supplemented animals were fed a diet containing 5mg/kg of Mo; the basal diet contained 4mg/kg of copper and 0.1mg/kg of Mo. The level of dietary Mo required to induce an infertility effect between these two values is not known however.

Relationship between serum copper and ferroxidase in cattle

Ferroxidase is a cuproenzyme containing eight atoms of copper per molecule. Since about 80% of the total plasma copper is present in the ferroxidase molecule highly significant correlations exist with plasma, serum and whole blood copper. As the amount of copper made available to the body becomes depleted, metabolic processes involving copper, e.g. ferroxidase activity, must compete for copper, and thus cuproenzymes are depleted. Essentially determining the activity of ferroxidase in serum provides the same information on the animals copper status as measuring elemental copper in serum. It does have some technical advantages as it is simpler to measure and is not affected by contamination with copper. The regression equation between serum ferroxidase and serum copper has been determined by the Animal Health Laboratory Network. This equation was used to derive the serum ferroxidase reference range.

Like glutathione peroxidase, ferroxidase is an enzyme whose activity is very much affected by the substrate, pH and temperature of the reaction. As a consequence ferroxidase reference ranges can not be extrapolated direct from the literature. The separate laboratories within the Animal Health Laboratories of MAF Quality Management use the same method so their results are directly comparable.

Conclusion

Milk production increases and fertility responses can occur following copper supplementation in dairy herds but at this stage there are too few trials published where a positive response has occurred to provide a firm reference range. The information to date does suggest that the reference range published for young growing cattle is suitable, especially for liver.

It is important to note however that there appear to be factors other than just the level of copper in serum or liver that influences the likelihood of a response. In the Morrinsville/Te Aroha study the herd with the lowest equal liver and serum copper concentration did not respond to supplementary copper in either of the two years. One of these factors is the level of molybdenum in the diet which stresses the importance of sampling the diet as part of any investigation into a possible copper deficiency problem.

COPPER REFERENCE RANGES - SHEEP

The manifestations of copper deficiency described in sheep include:

Enzootic ataxia
osteoporosis
suppressed lamb growth
suppressed wool growth
achromotrichia and steely wool
suppressed fertility in ewes and rams
anaemia.

The Ministry of Agriculture and Fisheries have a published production related reference range for liver but not serum copper[23]. Liver copper levels <95µmol/kg WM are considered responsive. The literature review in the table supports this value in 9 of the 11 studies. In the two reports[24,25] where the liver copper levels were above this the molybdenum intake was quite high (up to 20mg/kg). These intakes are much higher than is generally found under field conditions and part of the responsive condition may in fact have been the direct effect of these high molybdenum intakes. Although such high intakes are rare in the field, it is important to determine the level of dietary Mo, S, Fe and Cu as this assists the interpretation of blood and tissue copper levels. As in cattle, copper deficiency induced by high Mo and S intakes appears to be more detrimental on animal performance than if due to low copper intakes alone[26].

MAF recommend that liver copper be analysed in sheep rather than serum copper. The main reason for this is that serum is an insensitive indicator of copper status. Normal serum copper levels (>11µmol/l) may be found in sheep with liver copper levels ranging from deficient to close to toxic levels (88-3000µmol/kg)[27]. As Table 2 indicates it is only when serum copper levels are low (<11µmol/l) that there is a close relationship with liver copper.

Table 2: The relationship between serum copper and liver copper in sheep[27].

No. Sheep	Serum copper* (µmol/l)	Liver copper (µmol/kg WMB)			
		<110	110-220	220-800	>800
47	<11	98%	2%	0%	0%
69	11-21	6%	23%	39%	32%

* Reported as whole blood copper but the concentration of copper in serum is the same as whole blood in sheep (RAHL data unpublished)

Serum copper analyses will therefore miss many sheep of low copper status; hence liver is the sample of choice especially if copper is being checked for preventive reasons. If serum is measured, then 11µmol/l is suggested as the decision level. A response to copper supplementation at serum concentrations greater than this is unlikely (refer to Table 3).

The effect of breed on copper status in sheep

The breed of sheep has a significant effect on its copper status. This has been extensively studied in Britain with Scottish Blackface, Cheviot and Welsh Mountain breeds and their crosses[28,29,30]. Of these breeds the Scottish Blackface is the most susceptible to copper deficiency and the Welsh Mountain is the most resistant due to genetic differences in the ability to absorb copper from the diet. Fortunately studies to date suggest that genotype does not change significantly the level of copper in liver or serum at which a response can be expected[30]. However it does mean that on a farm where there are multiple breeds, the copper status of each breed needs to be assessed separately as it is possible that one breed is deficient and another quite adequate. The relative susceptibility of the common New Zealand breeds of sheep has not been investigated to the authors knowledge.

Conclusion

1. **Liver is the sample of choice for determining the copper status of sheep. Aim for levels greater than 95µmol/kg.**

2. **If there is more than one breed on a farm determine the copper status of each breed separately.**

3. **As part of the investigation, sample feed to measure molybdenum, sulphur, iron and copper levels.**

Table 3: Serum and Liver copper levels of unsupplemented sheep in copper responsive conditions.

RESPONSIVE CONDITION	BREED	Serum Cu µmol/l	Liver Cu µmol/kg WM	COMMENTS	REF
Growth rate	Romney	N.M.*	N.M.	New Zealand field study	31
	Romney	11	190	New Zealand field study. High Mo intake	24
	Scottish Blackface	3.14	20	British field study. High Mo intake.	32
	Suffolk x Shropshire	17	59	Experimental using high Mo diet.	33
	Scottish Blackface	7.9	N.M.	Experimental using high Mo/S±low Cu diet	34
	Suffolk x Mule	N.M.	N.M.	Intravenous tetrathiomlybdate	35
Fleece Weight ± achromotrichia	Romney	N.M.	N.M.	New Zealand field study	31
	Romney	11	190	New Zealand field study. High Mo intake	24
	Merino	6.3	N.M.	Aust. field study. Mo intakes varied from 1.2-8 mg/kg.	36
	Scottish Blackface	3.14	20	British field study. High Mo.intake.	32
Ewe fecundity	Romney	N.M.	N.M	New Zealand field study.	31
	Scottish Blackface	<6	47	Scottish experimental study. Signs most notable on high Mo/S	26
	Suffolk x mule	N.M.	N.M.	Intravenous tetrathiomolybdate	35
Bone changes	Romney	11	190	New Zealand field study. High Mo.intake	24
	Scottish Blackface	3.14	20	British field study. High Mo.intake.	32

Table 3 continued

RESPONSIVE CONDITION	BREED	Serum Cu µmol/l	Liver Cu µmol/kg WM	COMMENTS	REF
Swayback	Mixed	N.M.	76±17 13-36 10-112 9-66 <45	Values from clinically affected lambs.	37 38 39 40 41
	Cheviot x Border Leicester	<3.14	<45	Longterm copper depletion with a low copper/low Mo diet.	42
	Scottish Blackface	<6	47	Scottish experimental study. Signs most notable on high Mo/S	26
Anaemia	Scottish Blackface	3.14	20	British field study. High Mo. intake	32
	Merino	6.3	N.M.	Aust. field study. Mo intakes varied from 1.2-8mg/kg.	36
	Scottish Blackface	<6	47	Scottish experimental study. Signs most notable on high Mo/S	26
	Scottish Blackface	7.9	N.M.	Experimental using high Mo/S±low Cu diet	34
Ram fertility	S.African Merino	12	143	Experimental using high Mo±S diets	25
	Mixed	4.4	N.M.	Serum Cu correlated with first service conception rate	43

* N.M.: Not measured

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