Alpaca trace mineral reference intervals

Data on trace mineral levels normally found in healthy alpaca grazing NZ pasture is sparse. The New Zealand Alpaca Association, with funding contributions from the MAF Sustainable Farming Fund and Gribbles Veterinary, undertook a project to expand this database (2007-2008).

Method:

Farmers were enrolled in this project if they were willing to have five healthy female adult alpaca bled for mineral testing. The age range was between 1 and 10 years and the definition of healthy was if both the farmer and veterinarian taking the samples considered them clinically normal.

Farmers were encouraged to sample the same alpaca four times in the year to represent spring (August to November), summer (December to February), autumn (March to May) and winter (June, July). This was to determine if there were seasonal variations in mineral levels.

Both clotted and EDTA blood was collected from the jugular vein and sent to the local Gribbles Veterinary lab. In most cases, samples were received at the laboratory within 24 hours of collection. Gribbles Veterinary tested the serum for copper (Cu), selenium (Se) and vitamin B12 and whole blood for selenium.

Selenium in serum and whole blood was determined using a semi-automated fluorimetric method.[1] A chemiluminescent method was used to assay vitamin B12 (Diagnostic Products, USA). Serum samples were digested with perchloric acid and assayed by atomic absorption spectrophotometry to measure their copper levels.

Results were recorded in Microsoft Excel and plotted according to analyte value, one plot per season. If the results appeared normally distributed, then the arithmetic mean and standard deviation (SD) were calculated. If the data was not normally distributed, it was log transformed and mean and SD recalculated. Any result outside three SD units was considered an outlier. These outliers were removed and the mean and SD recalculated.

Results:

A total of 15 alpaca farms participated in this study. Eleven of these farms sampled alpaca in spring, three in summer, five in autumn and ten in winter. Samples from all four seasons were received from just one farm. Three farms sampled three times, five farms sampled twice and six farms sampled just once. The alpaca farms were scattered throughout both islands. Too few farms were sampled three or four times through the year for an assessment of seasonal variation to be made.

The farm data from the first bleeding was used for the reference interval study. This means the reference interval data was derived from spring (11 farms, 53 animals), autumn (one farm, 5 animals) and winter (three farms, 13 animals). Each farm sampled 5 animals except for 4 on two farms and 3 on one farm. In total, serum Se was measured in 69, serum Cu in 70, and serum B12 and whole blood Se in 71 alpaca.

The data is summarized as frequency distribution graphs in figures 1-4 (see end of document). The reference intervals in table 1 are the 95% confidence intervals for the different analytes (expressed as 2.5 and 97.5 percentiles).

Both serum and whole blood Se concentrations were measured in the alpaca. The same samples were used to determine the regression equation between blood Se and serum Se as were used for the reference interval study (15 farms, 69 animals). The relationship is represented graphically in figure 5. The regression equation is y=1.054x + 144.6 where

y = blood Se (nmol/L) and x = serum Se (nmol/L). The correlation coefficient (r^2) is 0.86. There was no statistically significant difference in the relationship between blood Se and serum Se between the four seasons.

Table 1:   Reference intervals for four minerals from healthy adult alpaca

Analyte	Units	Lower	Upper
Serum Cu	umol/L	5.2	16
Serum B12	pmol/L	70	880
Serum Se	nmol/L	240	2400*
Blood Se	nmol/L	350	2800*

* Log transformation did not completely normalize the distribution of serum Se or blood Se. The 97.5 percentile for both these analytes was a lot higher than the highest measurement in the study. The values quoted here are the highest values recorded in this study.

Discussion:

The reference intervals used in New Zealand for mineral levels in sheep and cattle are generally production related.[2] By using these intervals, it is possible to predict whether a group of animals will give a production response to supplementation by comparing their mean analyte level with the reference interval. These ranges have been developed as a result of many production trials over the years which are a costly and time consuming process.

When new production animal species, like alpaca are introduced into New Zealand, it is generally not possible in the short term to develop production related reference intervals because the industry is small and the level of financial investment required is quite high. However, there is still a need for a benchmark of mineral levels to be developed to help veterinarians interpret blood and liver mineral levels. Although production related reference intervals are the ideal, useful information can be gathered by establishing the range of mineral levels in clinically normal productive animals grazing typical NZ pasture. It can be assumed that if an animal's levels are within such a range, then they will not be severely deficient or at risk of severe toxicity.

The intervals established from this study will be valuable for the alpaca farmers and their veterinarians. Using these ranges, they will be able to determine if a mineral deficiency is a possibility on their property and will have ranges against which they can monitor the effects of any supplementation that is advised. If levels stay within these ranges, then the risks of severe deficiencies or toxicities developing are low.

A correlation coefficient of 0.87 between serum and whole blood selenium is quite high and suggests that both analytes are useful indicators of an alpaca's selenium status. In cattle, serum selenium levels change more rapidly than whole blood selenium when dietary selenium intakes change and this has led to the widespread adoption of the serum test in cattle herds that are on supplementation programmes.[3] Some of the alpaca that were enrolled in this study were being supplemented with selenium and others not. This information was not collected and evaluated in this study so it is not possible to determine if the relationship changes with supplementation in alpaca as it does in cattle.

The distribution of serum B12 levels was markedly skewed to the right. This was corrected using log transformation. Even though the other analytes were not so skewed, log transformation resulted in a more guassian distribution for these as well. Both serum and blood selenium, however were still sufficiently skewed after log transformation that the 97.5% percentile value had to be replaced by the highest value in the study.

Table 2 compares the results of this study with a similar alpaca study from Australia and the reference intervals currently used for cattle in New Zealand.

Table 2: Comparison of reference intervals for NZ and Australian alpaca and NZ cattle

Analyte	Units	Alpaca (NZ)	Alpaca (Aust)[4]	Cattle
Serum Cu	umol/L	5.2 – 16	7.6 – 10.2	8.0 – 20
Serum B12	pmol/L	70 – 880	146 – 416	>100
Serum Se	nmol/L	240 – 2400		150 – 1000
Blood Se	nmol/L	350 – 2800	1330 – 1890	250 – 2000

The alpaca ranges from Australia are not that dissimilar to the production related reference intervals used for NZ cattle. Serum Cu levels are generally lower but even in cattle; individual animals can be as low as 6.5 umol/L without showing any production effects. This is because the production related reference intervals used in cattle are based on means of the group being tested and individual animals can have levels below these ranges, as long as the mean is within the range.[2]

In general, the Australian ranges are narrower. They sampled five alpaca herds compared with 15 in this study, which could be the explanation for the reduced ranges. Another difference is that the low end of the range for blood Se was considerably higher than the low end of the range in the NZ study. This occurred despite some of the pasture in the Australian study having relatively low selenium levels (0.03 ppm). Although not stated in the paper, the likely explanation for this difference is that all the alpaca in the Australian study were being supplemented with selenium whereas only some of the alpaca in the NZ study were.

It was decided to use a sample number of 5 for this study for two main reasons:

- This is similar to the recommended sample numbers for cattle testing (serum Se = 4, whole blood Se = 4, serum Cu = 6, serum B12 = 5)

- Most alpaca herds are small with 80% being under 20 animals. Five animals therefore represent a significant proportion of most herds.

In cattle and sheep, the following formula is used to calculate the appropriate number of animals to sample. This formula indicates that sufficient animals should be sampled to make sure the difference between the sample mean and the true mean (if all animals in a group were sampled), is no more than half the marginal range in 90% of cases.[5]

n (sample number) $\geq s^2 \times 1.65^2$ / (half the marginal range).[2] Where s = standard deviation of individual values within the sample and 1.65 corresponds to the 90% probability level in a

standard normal distribution. The estimation of s is based on large numbers of measurements being taken (at least 100 groups).

It is not possible from this study to verify if a sample number of 5 is appropriate, as only 15 groups were sampled (i.e. the estimate of s is not robust) and we don't know the size of the marginal ranges. However, the estimates for s for serum Cu and vitamin B12 in this study did suggest that five samples are likely to be appropriate for these analytes. In contrast, the between animal variability for both serum Se and blood Se was unexpectedly large and 5 samples may not be enough, particularly in larger herds. A possible explanation for this could relate to different levels of selenium supplementation between animals within herds. Because of the small herd sizes, and the close association of each animal with its owner, it is possible that some animals on a farm are being supplemented for various reasons whereas others are not. One technique to reduce the effect of this large within group variation is to sample the same animals each time and compare levels of the same animals over time.
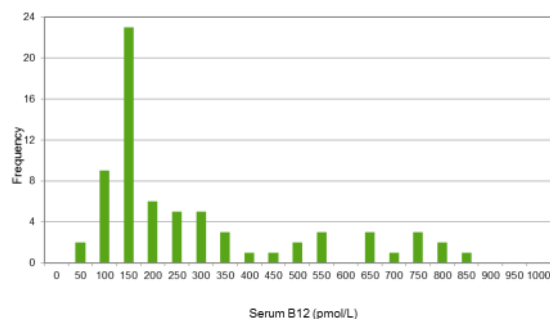
Figure 1: Frequency distribution for serum selenium
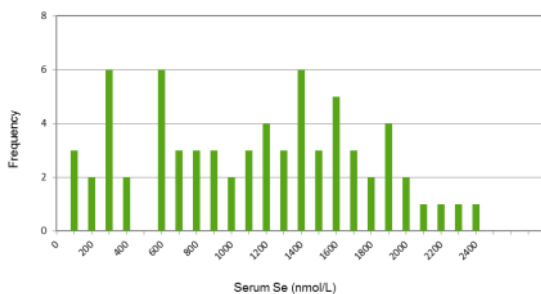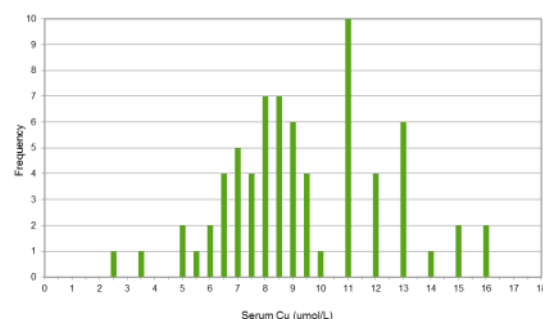

Figure 2: Frequency distribution for blood selenium
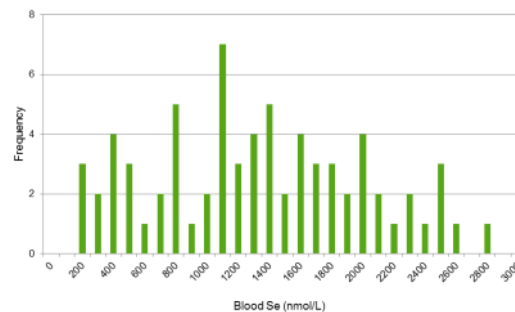
Figure 3: Frequency distribution for serum B12

Figure 4: Frequency distribution for serum copper

References:

1. Watkinson JH. Fluorometric determination of selenium in biological material with 2,-3 diaminonaphthalene. *Analytical Chemistry* 38:92-7, 1966
2. Fraser AJ. Production related reference intervals. Laboratory diagnosis of trace element deficiency disease. *Surveillance*, 9:4-7, 1982
3. Thompson KG, Fraser AJ, Harrop BM, Kirk JA, Bullian J, Cordes DO. Glutathione peroxidase activity and selenium concentration in bovine blood and liver as indicators of dietary selenium intake. *New Zealand Veterinary Journal* 29:3-6, 1981
4. Jusdon GJ. Survey of the mineral, trace element and vitamin status of alpacas. *Camelid Medicine and Surgery*, Proceedings 278, Post Graduate Foundation in Veterinary Science, University of Sydney, Pp 311-317
5. Fraser AJ. How many samples? Laboratory diagnsis of trace element deficiency disease. *Surveillance* 9:7-9, 1982

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