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Comparison of Trace Mineral Concentration in the Various Lobes of the Liver of Alpacas and Llamas

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Abstract: The objective of this study was to determine if the site of liver tissue collection would effect analysis of selected trace minerals in the liver of alpacas and llamas. Randomized post-mortem study. Livers were collected during post-mortem examination of alpacas and llamas that died of various diseases. Liver tissue was collected from the left, rightand caudate lobes of the liver for analysis. Trace mineral analysis was performed at a commercial laboratory using inductively coupled argon plasma emission spectroscopy. Significant differences were not found among sites of tissue collection except for potassium. Coefficients of variation were > 50% for calcium, copper, iron, molybdenum, seleniumand zinc. Site of liver tissue collection does not significantly effect trace mineral analyses in alpacas and llamas.

Key words: Alpaca, Liver, Llama, Trace Minerals

Introduction

Llamas and alpacas have become commonplace in many areas of North America and Australia and are gaining popularity in many areas outside of South America. These camelids are intensively managed as breeding and fiber producing animals, but are also used as pets, beasts of burdenand show animals. Owners are actively involved in assessment of adequacy of diet for general health and optimum productivity. To date, few studies have been performed to establish references for trace minerals in these species (Hasting and Gascoyne, 1992 and Espinoza *et al.*, 1982). Differences in soil, available forageand water resources may effect trace mineral absorption and metabolism. Therefore, veterinarians often are asked to evaluate trace mineral status of llamas and alpacas residing on individual farms in addition to simply performing diet analysis.

Serum frequently is used to determine trace mineral status because venous blood is easily obtained, the procedure is minimally invasiveand the risk of complications is low. (Simons *et al.*, 1993; Dart *et al.*, 1996 and Bengoumi *et al.*, 1998). Serum and whole blood often are poor samples to evaluate nutritional status or changes in nutritional plane. Liver tissue analysis is considered more accurate for determination of trace mineral status. Collection of liver tissues often is done during post-mortem examination of llamas and alpacas that have died. More recently, percutaneous liver biopsy has been suggested for small sample trace mineral analysis (Anderson *et al.*, 1998). Percutaneous samples are obtained from the right lobe of the liver. However, accidental biopsy of the caudate lobe may occur and ultrasound guided biopsy of the left lobe of the liver is possible. Trace mineral storage in the liver may not be uniform and variations have been found in cattle and sheep. (Chapman *et al.*, 1970 and ,Chapman *et al.*, 1970) Variations in liver copper storage in sheep was hypothesized to occur because of variable portal blood flow to various lobes of the liver (Heath and Perkins, 1985). Although variations in copper concentration was observed in horses, these differences were not statistically significant (Pearce *et al.*, 1997 and Cymbaluk *et al.*, 1986) The hypothesis for this study was that the site of sample collection would have no effect on trace mineral analysis. Therefore, the purpose of this study was to compare the concentration of commonly analyzed trace minerals in the left, rightand caudate lobes of the liver of llamas and alpacas.

Materials and Methods

Sample Collection - Complete livers were collected randomly from alpacas and llamas during post-mortem examination. Tissues from the left, rightand caudate lobes of each liver were collected, individually labeledand frozen at -20 C until trace mineral analysis was performed. Liver samples from the left and right lobes were collected from the middle portion of the lobes. The caudate lobe was identified as a small projection from the visceral aspect of the liver on the dorsal junction of the right and left lobes. This lobe was removed and used for analysis.

Trace Mineral Analysis - Frozen liver tissue samples were mailed on ice overnight to the testing laboratory (Michigan State University Diagnostic Laboratory, East Lansing, MI, USA). Trace mineral analyses were performed by inductively coupled argon plasma emission spectroscopy (Braselton, personal communication, 1999). (Stowe *et*

al., 1985 and Braselton *et al.*, 1981). Trace mineral analysis included calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, sodiumand zinc.
Statistical Analysis - Repeated measures analysis of variance was used for data analysis and Duncan's test used for post-hoc testing if indicated. Significance was pre-set at $P<0.05$.

Results

Liver specimens were obtained from 5 alpacas and 5 llamas that had died of various diseases. Summary data for trace mineral analyses are presented in Table 1. Statistical analyses were not performed for samples in which the mineral concentration was below the detection limit of the test. Trace mineral data that was not analyzed for this reason included cobalt, 6 of 10 livers for seleniumand 3 of 10 livers for molybdenum. Although variations were observed between liver sample locations for all elements, no statistical differences were found except for potassium. Repeated measures analysis of variance found significance for potassium ($P=0.049$), but post-hoc testing failed to identify a difference between individual sites. The coefficient of variation (CV) was > 50% for calcium, copper, iron, molybdenum, seleniumand zinc.

Discussion

Based on the results of this study, the site of liver sample collection does not effect the interpretation of trace mineral analysis for calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, selenium, sodium, or zinc for llamas and alpacas. Although not statistically significant, variation was found among sites which was similar to that which has been reported for sheep, cattleand horses. (Chapman *et al.*, 1970;Chapman *et al.*, 1970; Pearce *et al.*, 1997 and Cymbaluk *et al.*, 1986) Although liver specimens from llamas and alpacas are most commonly collected during post-mortem examination, percutaneous liver biopsy has become more routine to determine if trace mineral deficiency or toxicity exists in the affected camelid or to gain insight into the dietary adequacy of the herd (Anderson *et al.*, 1998). This study was not designed to establish reference ranges for trace mineral determination and the ranges given should not be used for assessment of adequacy of nutrition. Livers were harvested postmortem so that accurate collection of liver specimens could be conducted. The caudate lobe of the liver of camelids is quite small and difficult to identify using ultrasonography, as has been reported for horses. Thus, the data are useful for comparison of site of sample collection, but not for clinical application of the values because diet, season, genderand disease process were not controlled.
In horses, variations in copper concentration were found and the coefficient of variation ranged from 2.9 to 46.37 % (Pearce *et al.*, 1997 and Cymbaluk *et al.*, 1986) Liver concentration of copper but not zinc or manganese, were found to be different among horses fed various diets. Chapman et al compared 4 biopsy sites in cattle and found that site of biopsy was a significant factor for copper concentration but did not effect iron or molybdenum determinations (Chapman *et al.*, 1970). Liver concentration of copper in sheep and cattle was highly variable within livers with coefficient of variation of 8.08 to 42.31 % for sheep and 7.49 to 53.46 % in cattle (Chapman *et al.*, 1970 and Chapman *et al.*, 1970) Variation in copper storage by the liver of sheep has been hypothesized to be caused by differential portal vein blood flow to the various lobes of the liver (Heath and Perkins, 1985). The inference from these studies is that composite liver samples or the means from multiple liver samples would most accurately represent hepatic trace mineral content. This study appears to be the first to evaluate the effect of site of sampling on trace mineral determination in liver tissue of camelids. The coefficient of variation was large for many of the minerals for which analyses are commonly done. This should serve to emphasize the probable roles of forages, supplemental feeds, water, soiland disease processes on liver trace mineral content.
Little information is available concerning nutritional requirements for trace minerals by camelids.[1,2] Espinoza *et al* performed surveys of forage, soiland livers of sheep and llamas in the native range of the llama.[2] This study found that forages were markedly below a selected critical level in phosphorus, sodium, seleniumand zinc. The liver mineral concentration in llamas of Co, Cu, Mnand Mo were higher, that of Fe lowerand that of Mg, Seand Zn similar to that of sheep. These results suggest that species differences exist in the ability to extract trace minerals from forages and / or in the storage of these minerals. Also, males were found to have significantly higher concentrations of Cu compared with females suggesting gender differences in the ability to extract from the diet or store in the liver certain trace minerals. Copper toxicity has been reported to occur in llamas (Junge and Thornburg, 1989). The author's found that affected llamas had liver copper concentrations ranging from 847 to 1700 ppm dry weight basis. These llamas had been fed a diet containing approximately 25 to 36 ppm copper. However, I have observed camelids with liver copper concentrations exceeding 800 ppm dry weight basis to remain clinically normal and with normal hematology and serum biochemistry values (Anderson DE, Unpublished data, Ohio State University 1998). Stress or other;22:733-737.

Table 1: Trace Mineral Analyses for Left, right and caudate lobes of the liver of ten llamas and aplacas

Element	N mean ± SD	Left mean ± SD	Right mean ± SD	Caudate mean ± SD	Composite mean ± SD	CV %
Calcium (ppm)	10	703 ± 1359	998 ± 2148	800 ± 1559	834 ± 1666	200
Copper (ppm)	10	96 ± 132	95 ± 129	107 ± 157	99 ± 135	136
Iron (pp,)	10	174 ± 222	142 ± 170	167 ± 170	161 ± 183	114
Magnesium (ppm)	10	183 ± 34	192 ± 50	186 ± 40	187 ± 41	22
Manganese (ppm)	10	2.0 ± 0.4	2.1 ± 0.3	2.0 ± 0.4	2 ± 0.4	18
Molybdenum (ppm)	07	1.4 ± 1.1	1.2 ± 0.8	1.4 ± 1.0	1.3 ± 0.9	72
Phosphorus (ppm)	10	3027 ± 756	2968 ± 1564	2864 ± 563	2956 ± 1032	35
Potassium (ppm)	10	2192 ± 302	2357 ± 303	2165 ± 357	2238 ± 322	14
Selenium (ppm)	04	4.8 ± 4.5	5.7 ± 4.6	5.7 ± 5.7	5.4 ± 4.5	84
Sodium (ppm)	10	1500 ± 321	1460 ± 335	1543 ± 393	1504 ± 340	23
Zinc (ppm)	10	37 ± 23	37 ± 23	35 ± 19	36 ± 21	58

8. O'Cuill T, Hamilton AF, Egan unknown factors may be important in the precipitation of mobilization of copper from the liver resulting in toxicity in camelids.

To our knowledge, determination of the minimum needs and maximum tolerance concentrations of trace minerals in camelids have not been done. This research will be necessary to accurately assess the significance of liver trace mineral analyses. Although composite liver samples may be preferable, based on the results of this study the site of liver tissue collection will not effect the interpretation of trace mineral data.

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