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Multi-Elemental Analysis of Bovine Liver Biops and Whole Liver

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Summary

Five, single parity MARC II cows were biopsied, slaughtered, liver recovered and used to compare the element concentration of the liver biopsy with the concentration of that site, post-slaughter and the concentration in the entire liver. Each liver was cut into 20 pieces, homogenized and a 1 g sub-sample analyzed for element concentration using an inductively coupled argon plasma emission spectrophotometer. Element concentrations were different between animals, but element concentrations between sites within a liver were only different for Mn, and Mg. The two regions of the liver that accounted for this difference were regions caudal and cranial to the portal vein on the lateral side. These regions also tended to be higher for Zn, Cu and Fe. The pre-slaughter liver biopsies over-estimated the concentration of Fe, Na, and Ca, and underestimated Zn, Cu, Mn, Mg and P compared to the post slaughter analysis of the same site. The results indicate the concentration of trace elements in liver tissue obtained at slaughter depends on the location from which the tissue was removed. The site used for liver biopsies in this study, may not represent the highest concentration of elements in the liver of the live animal.

Introduction

Liver biopsies are used to assess trace element status of cattle for diagnostic and experimental purposes and generally provide better information than serum samples. Liver tissue collected at necropsy or slaughter is also used. The liver usually serves as the storage site for minerals. Serum concentrations may be maintained within adequate concentration ranges, at the sacrifice of liver mineral stores. If animals are receiving insufficient mineral, but have not yet depleted their liver stores enough to significantly lower the serum concentration, analysis of serum will be misleading. But analysis of liver biopsy samples may detect low liver trace element status.

Most published data used to establish the adequate liver mineral concentration ranges were collected from livers under experimental conditions at necropsy or slaughter. Few comparisons of trace element concentrations in liver biopsy and whole liver samples are available. There are no published mineral concentration ranges based upon liver biopsy samples. The objectives of this study were 1) to determine if trace element concentrations in the liver depended upon the liver section from which the sample was collected, 2) to compare the trace element content of pre-slaughter liver biopsy samples with post mortem trace element concentrations of the entire liver, 3) to determine if the magnitude of the concentration differences was sufficient to effect interpretation of nutritional status and 4) to determine if any gross affect was evident in livers from which previous biopsy samples had been collected.

Procedure

Five, single parity MARC II females, which had previously been

liver biopsied four times, were biopsied again, and then slaughtered five hours later. Pre-slaughter liver biopsies were removed from the most caudal region of the liver from between the twelfth and thirteenth rib, 20 cm ventral to the mid-line using a Tru-Cut® biopsy needle. A total of five to eight consecutive biopsy cores were obtained on each cow and weighed approximately .1 g (wet weight). The entire liver was recovered at slaughter and iced. The liver was marked into a grid using repeatable structures on the liver, cut into 20 sections (Figure 1), and stored frozen until analyzed. Frozen liver sections were thawed and blended using a Waring blender until liquidous. A 1 g subsample was removed and digested in concentrated nitric acid overnight. Four pieces were randomly selected from each liver for duplicate analysis. The digests were diluted and analyzed for calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), phosphorus (P), and zinc (Zn), using an inductively coupled argon plasma atomic emission spectrometer equipped with an ultrasonic nebulizer.

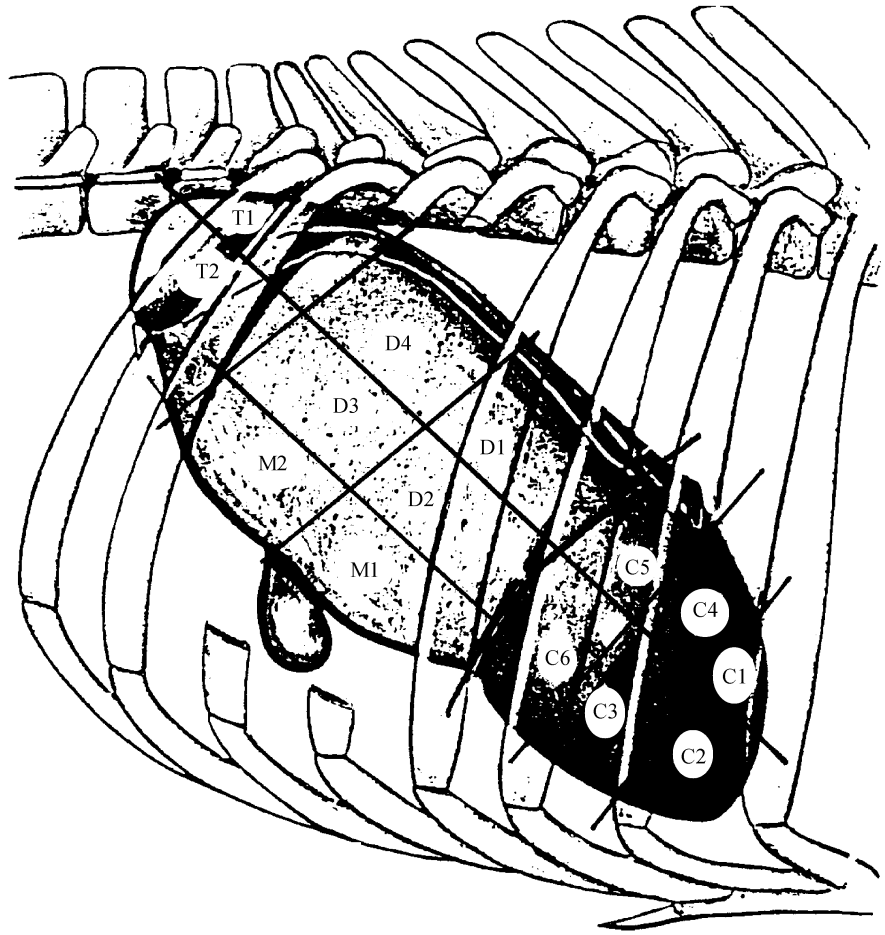

Figure 1.

Results

Due to normal biological variation between animals, element concentrations were different between livers ($P < .05$) for all elements except P and Ca ($P > .05$). Examples of the differences detected for Cu, Zn, and Mn are shown in Table 1. The reason Ca and P concentrations did not differ may be because the bone, not liver serves as the primary storage site for Ca and P. Therefore, liver concentrations of both elements are similar between animals. Furthermore, Ca homeostasis is vital to life, and biological variation may be less than other minerals.

Significant differences in concentration by site within liver were detected only for Mn, and Mg ($P < .05$). Two regions of the liver, designated D1 and D3, consistently were found to contain higher concentrations of Mn, Mg, and Mo than other regions of the liver (Table 2). Regions D1 and D3 were caudal and cranial to the portal vein on the lateral side, respectively. Those regions also tended to be higher for Zn ($P = .06$), Fe ($P = .11$) and Cu ($P = .09$).

The liver generally serves as a significant storage site for many elements. Liver cells (hepatocytes) actively absorb them from the blood stream. Regions nearer the portal vein,

(Continued on next page)

Table 1. Means for trace element concentrations of the entire liver, by animal (mg/kg, wet weight).

Animal	Cu	Zn	Mn
1	41.7[a]	38.7[a]	2.32[a]
2	67.8[b]	61.3[c]	2.55[ab]
3	53.3[a]	38.1[ab]	2.71[b]
4	62.3[b]	33.2[b]	2.31[a]
5	46.6[a]	42.5[a]	3.18[c]
S.E.	2.5	1.82	.12

[abc]Means in a column with different superscripts are different ($P < .05$).

Table 2. Comparison of means of trace element concentrations of sites D1 and D3, caudal and cranial to the portal vein on the lateral side, with the mean value of the entire liver (mg/kg, wet weight).

Element	Mean	D1	D3	SE	P-value
Cu	52.6[a]	65.3[b]	68.3[b]	5.0	P = .09
Zn	41.2[a]	57.2[b]	52.7[b]	3.7	P = .06
Mn	2.55[a]	3.65[b]	3.44[b]	.23	P = .05
Fe	44.9[a]	55.3[b]	54.4[b]	3.8	P = .11
Mg	190.4[a]	252.2[b]	239.8[b]	13.3	P = .01
Mo	.97[a]	1.18[b]	1.17[b]	.08	P = .15
Ca	44.8[a]	61.7[b]	53.1[b]	5.8	P = .08
Na	696.2[a]	795.2[b]	743.8[b]	53.7	P = .30

[ab]Means in a column with different superscripts are statistically different.

Table 3.Trace element concentration mean and range for the liver of Cow 2 (mg/kg, wet weight).

Element	Mean	S.E.	Low	High	Adequate[1].
Cu	68.3	12.0	32.6	99.9	25-100
Zn	63.0	10.9	42.1	111	25-100
Mn	2.60	.51	1.43	4.62	2-6
Fe	46.0	7.3	37.4	75.0	45-300
Mg	202	25	161	310	100-250
Ca	43.3	9.2	32.8	75.1	30-200
Na	740	153	529	1210	530-3450

[1] Puls (1994).

Table 4. Mean comparison of trace element concentrations of the biopsy (pre-slaughter) with the same site in the liver (T1 and T2) post-slaughter (mg/kg, wet weight).

Element	Biopsy	T1	T2	S.E.
Cu	20.9[a]	62.9[b]	56.1[b]	5.77
Zn	22.9[a]	41.0[b]	41.8[b]	1.9
Mn	1.45[a]	2.40[b]	2.61[b]	.16
Fe	107[a]	43.2[b]	45.4[b]	10.0
Mg	127.2[a]	184.8[b]	191.8[b]	7.61
Ca	62.5[a]	45.6[b]	45.7[b]	5.3
Na	2600[a]	666[b]	720[b]	248

[ab]Means in a row with different superscripts are different (P < .05).

entrance into the liver, may contain blood with higher concentrations of elements. Consequently, hepatocytic uptake of elements in those regions may be greater than it is in regions "down stream," because in those regions blood contains a relatively lower element content. Higher concentrations of most elements found in regions designated D1 and D3 may be related to blood flow through the liver.

To illustrate the variation of element concentrations within the liver, Cow 2 was selected at random. The mean, lowest and highest concentrations for all trace minerals found in that liver are listed in Table 3. We found that trace element concentration does vary within the liver. Assessment of trace mineral status based upon an analysis of a sample from one section of liver could lead to erroneous conclusions. Table 3 also compares the highest and lowest mineral concentrations found in Cow 2 to the adequate concentration ranges reported by Puls (1994, Mineral Levels in Animal Health: Diagnostic Data). Some of the highest and lowest concentrations found fall outside of the adequate range. Assessment of trace mineral status based upon samples tending to contain higher or lower mineral amounts than the average may lead to erroneous conclusions, therefore, it is important that the section of liver from which a sample is taken be identified. Better interpretation of the results of analysis should be possible with such information. If assessment of possible trace element deficiency is desired, it is best to take a liver sample from the area near the portal vein at the top of the liver. If trace element concentrations are low in that area, then the animal is likely deficient.

Results of pre-slaughter liver biopsy sample analysis tended to overestimate the concentration of Fe, Ca and Na ($P < .01$), and under-estimated the concentrations of Zn, P, Mn, Mg and Cu ($P < .05$), compared to results from whole livers (Table 4). The areas designated T1 and T2 were the most distal region of the liver with T1 being dorsal to T2. Four of the five biopsies were removed from T1 and the remaining biopsy was from both T1 and T2.

Comparison of results from liver biopsy analysis to the normal concentration ranges derived from whole liver analysis should be made with caution. The concentrations detected in the biopsy sample will differ from mean whole liver concentrations and the amount of difference will depend upon the site from which the biopsy sample was taken. One possible reason for the difference between biopsy and post slaughter trace element content may be due to the relative amounts of blood contained in each. The animals in this study were exsanguinated at slaughter before their livers were removed. The difference found for Fe and Na is likely due to the difference in whole blood content of the biopsy and whole liver samples. The concentrations of Zn, P, Mn, Mg and Cu may have been increased, with respect to the liver biopsy samples, after exsanguination.

Biopsy sites were difficult to find even though biopsies were taken only five hours before slaughter. Close inspection of this section of liver revealed no significant lesions. Even dissection of the liver revealed no scaring from any of the biopsies. This indicates repeated biopsies from the same site does not cause any long term liver structure damage.

Conclusions

Liver biopsy samples can be used to assess the mineral element status of individual animals, but the site within the liver from which the biopsy sample was collected must be known for the best assessment.

Comparison of trace element content in liver biopsy samples to normal trace element ranges derived from whole liver data should be made with caution. Results from biopsy samples may be significantly higher or lower than mean whole liver concentrations. The site used for liver biopsies in this study may not represent the highest trace element concentrations in the liver of the live animal.

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