Analytical methodology

Variation in trace element content between liver lobes in cattle. How important is the sampling site?

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ABSTRACT

The aim of the present study was to establish the pattern of lobular distribution of trace elements in the liver of cattle. The objective was to determine which part of the liver would provide accurate estimation of the trace element content of the whole organ. Liver samples were obtained from 10 Holstein-Friesian (HF), 10 Galician Blond (GB) and 10 GBxHF crosses (all aged 10 months) at slaughter. Samples were taken from 6 regions of the liver: the internal and external faces of the right lobe (IR and ER respetively); the left lobe (L), caudate lobe (CAU), quadrate lobe (QUA) and the processus papillaris (PP). The samples were acid digested and trace elements were determined by inductively coupled plasma mass spectrometry. The distribution of all trace elements, except cobalt and zinc, varied significant across the liver. In all cases, the concentrations were highest in L and lowest in CAU. Variations in the distribution between the other areas of the liver (ER, IR, QUA, PP) were not significant. The distribution of trace elements may be related to oxygen perfusion. Moreover, the trace element content of CAU was weakly correlated with those of the other lobes, and the capacity of L to accumulate high levels of trace elements would only be observed at high levels of exposure. Taking into account the main findings of the study, a single sample of liver taken from the same anatomical region (excluding CAU and L) would be adequate for determining the trace element status of cattle.

1. Introduction

Trace minerals are required for the normal functioning of almost all biochemical processes in the body [1]. Trace element deficiencies are frequent worldwide and are closely associated with poor animal health and productivity; however, excessive exposure also has deleterious effects on health and production [2]. In addition, interactions between trace elements, particularly when present at high or unbalanced concentrations, largely determine physiological needs and are frequently involved in the pathogenesis of numerous disorders in farm animals (e.g. interaction between copper, sulphur and molybdenum, or between copper, iron and zinc in ruminants [1]).

Determination of trace element status is an essential diagnostic tool when problems associated with decreased productivity, low fertility and immunity appear in the herd. Within the non-invasive samples, measurement of trace element concentrations in blood (serum or plasma), can provide useful information about the trace element status, even though may not be possible to discriminate between deficient,

adequate or even excessive trace element exposure in some circumstances. For example, in some animals serum or plasma copper concentrations may be within the adequate range, but hepatic levels may be very low [3]. Similarly, copper deficiency may be diagnosed on the basis of low serum/plasma copper concentration and a toxic or near toxic state may be observed upon necropsy or hepatic biopsy [4,5]. In addition, trace element concentrations in blood are generally 1–100 times lower than in tissues (particularly the liver) and vary within a narrow range [3], which can make diagnosis imprecise, particularly in laboratories with limited experience on trace element analysis.

Within the body tissues, the liver is generally considered the best tissue for evaluating trace element status in livestock because it is a storage organ for most trace elements and its easy of sampling [1]. Although in vivo liver biopsy is a simple procedure with low associated risk, farmers are often reluctant to agree to their livestock being subjected to the procedure. In addition, the amount of sample obtained is small and may be contaminated by blood [6]. However, *postmortem* liver sampling at slaughter is assumed to be very useful for checking the

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herd mineral status and it has even been proposed for monitoring processes from the point of view of human nutrition (e.g. to monitor excessive copper accumulation in cattle: [7]). When liver samples are collected at slaughter it is important to bear in mind that trace elements will not be homogenously distributed across the whole organ. Although the available data on livestock is limited [6,8], an uneven distribution of trace elements has been demonstrated in the hepatic parenchyma in humans [9–11]. Recent studies also indicate possible differences in trace element metabolism between breeds depending on their aptitude, namely catabolic or secretion type (typical of milking breeds) or anabolic or accretion type (characteristic of the beef breeds) [12–14], which could lead to different patterns of hepatic distribution of trace elements. This adds further uncertainty to the analysis of hepatic concentrations of trace elements.

This study was conducted to establish any patterns in the inter-lobular distribution of trace elements in the liver of cattle and which may be at least partly related to the hepatic metabolism and/or may vary between breeds, depending on their aptitude. The overall objective was to determine which area of the liver would be more adequate to be used as a biomarker of the trace element status.

2. Material and methods

2.1. Liver samples

The liver samples were obtained from calves during a wider study to evaluate trace element status. The calves were reared in a commercial feedlot with a typical beef cattle diet based on a standard trace element-supplemented corn, barley and soybean meal concentrate feed (in mg/kg feed Co: 0.40, Cr: 2.91, Cu:22.1, Fe: 163, Mn: 101, Mo: 1.12, Ni: 2.12, Se: 0.249, Zn: 102) and barley straw (ca. 1 kg/animal/day). Detailed information about the animals and diets are reported elsewhere [14].

Briefly, samples of liver were taken from 10 male Holstein-Friesian (HF), 10 Galician Blond (GB) and 10 GBxHF crosses (all aged 10 months) at slaughter in a commercial abattoir. The liver was excised from the carcass immediately after slaughter, and samples of approximately 5 cm length × 3.5 cm width × 3.5 cm height were taken from 6 regions of the liver: the internal and external faces of the right lobe (IR and ER, respectively), the left lobe (L), caudate lobe (CAU), quadrate lobe (QUA) and the processus papillaris (PP). The ER is the lobe usually sampled in needle biopsy procedures [6,8].

2.2. Sample preparation and analysis

In the laboratory, liver samples were freed of connective tissue and major blood vessels and were then homogenized. Subsamples of approximately 2 g were digested in 5 mL of 69% nitric acid and 2 mL 33% w/v hydrogen peroxide in a microwave digestion system (Milestone, Ethos Plus). The digested samples were transferred to polypropylene sample tubes and diluted to 25 mL with ultrapure water. The concentrations of essential trace elements (Co, Cr, Cu, Fe, Mn, Mo, Ni, Se and Zn) were determined by inductively coupled plasma mass spectrometry (ICP-MS). An analytical quality control programme was applied throughout the study. Signal intensity values were monitored throughout the analysis and subtracted from the readings for calculation of the final values. The limits of detection (LoD) were calculated as three times the standard deviation of the reagent blanks; none sample was below these LoD. Analytical recovery, determined with certified reference material (1577c Bovine Liver, National Institute of Standards & Technology, USA) were 103.7% (Co), 96.2 (Cr), 99.2 (Cu), 99.1 (Fe), 100.3 (Mn), 100.6 (Mo), 11.9 (Ni), 98.4 (Se) and 100.2 (Zn).

2.3. Statistical analyses

All statistical analyses were carried out using SPSS for Windows (vs

Table 1

Summary of the general linear model analysis of trace element concentrations in liver, with breed and lobe as main factors.

	breed	lobe	breed*lobe	R^2
Co	$F_{2,179}$ = 7.511 ***	$F_{5,179}$ = 0.403	$F_{10,179}$ = 1.207	0.152
Cr	$F_{2,179}$ = 1.270	$F_{5,179}$ = 12.508 ***	$F_{10,179}$ = 1.023	0.317
Cu	$F_{2,179}$ = 12.324 ***	$F_{5,179}$ = 2.768 *	$F_{10,179}$ = 0.134	0.197
Fe	$F_{2,179}$ = 0.736	$F_{5,179}$ = 3.732 **	$F_{10,179}$ = 0.391	0.129
Mn	$F_{2,179}$ = 6.432 **	$F_{5,179}$ = 5.165 ***	$F_{10,179}$ = 0.519	0.213
Mo	$F_{2,179}$ = 17.121 ***	$F_{5,179}$ = 12.572 ***	$F_{10,179}$ = 0.216	0.380
Ni	$F_{2,179}$ = 0.213	$F_{5,179}$ = 3,184 **	$F_{10,179}$ = 0.756	0.191
Se	$F_{2,179}$ = 0.565	$F_{5,179}$ = 7.293 ***	$F_{10,179}$ = 1.518	0.246
Zn	$F_{2,179}$ = 4.774 **	$F_{5,179}$ = 0.719	$F_{10,179}$ = 0.028	0.077

* $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$.

21). The normality of data distribution was checked using the Kolmogorov-Smirnov test. A general lineal model (GLM) was used to test for interlobular and between-breed differences in trace element concentrations. Post hoc DHS Tukey tests were used to test for differences in trace element concentrations between hepatic lobes. Associations between trace elements and hepatic lobes were evaluated by Pearson's correlation coefficient at a significance level of $P < 0.05$.

3. Results and discussion

The results of the GLM used to evaluate the effect of lobe (IR, ER, L, CAU, QUA and PP) and breed (HF, GB and GBxHF) on trace element distribution in the calf livers are presented in Table 1. Both lobe and breed are significant factors in the analysis for most trace elements. No significant interactions between lobe and breed were found for any trace element, indicating that distribution of trace elements across the liver is the same in both breeds and their crosses. Consequently, in the present paper the calves data for all breeds are presented together.

Fig. 1 shows the trace element distribution within the different liver areas sampled. Overall, the trace element status of all calves included in the study was adequate (according to [3,15]) and only copper concentrations were above adequate for cattle. Statistically significant differences were found in the distribution of all trace elements, except for cobalt and zinc, across the liver. In all cases, the concentrations were highest in L and lowest in CAU. A similar pattern has previously been described for copper in cattle receiving high copper supplementation [8] and may be related to the proximity of the liver area to the central vessels. Oxygen perfusion may be greater in liver areas closer to central vessels (e.g. in CAU) and support higher metabolic activity related to the incorporation of trace elements into enzymes for export to tissues (i.e. caeruloplasmin or ferritin, for transport of copper and iron respectively), or for biliary excretion. By contrast, oxygen perfusion and metabolic activity may be lower in regions further from central vessels (namely L), which may consequently lead to higher trace element deposition. This pattern of trace element deposition within the liver (higher concentrations in L) may be particularly important in animals exposed to trace element concentrations above physiological needs, in which trace element homeostasis is largely maintained by biliary excretion [16]. A similar inter-lobular distribution (accumulation in L) has been described in human patients with copper (Wilson's disease) and iron storage diseases ([10,11], respectively). In our study, as in intensive farming in general [2], calves received mineral supplementation above the physiological needs, and it is therefore possible that the higher trace element concentrations in the left lobe may represent trace element stores (i.e. copper and zinc bound to metallothionein [17]). If our hypothesis were true, weaker or no differences between the left lobe and other liver areas would probably be observed in animals with a low trace element status. In fact, lower copper and iron concentrations have been observed in the left lobe than in other hepatic lobes in cattle with a low trace element status [6].
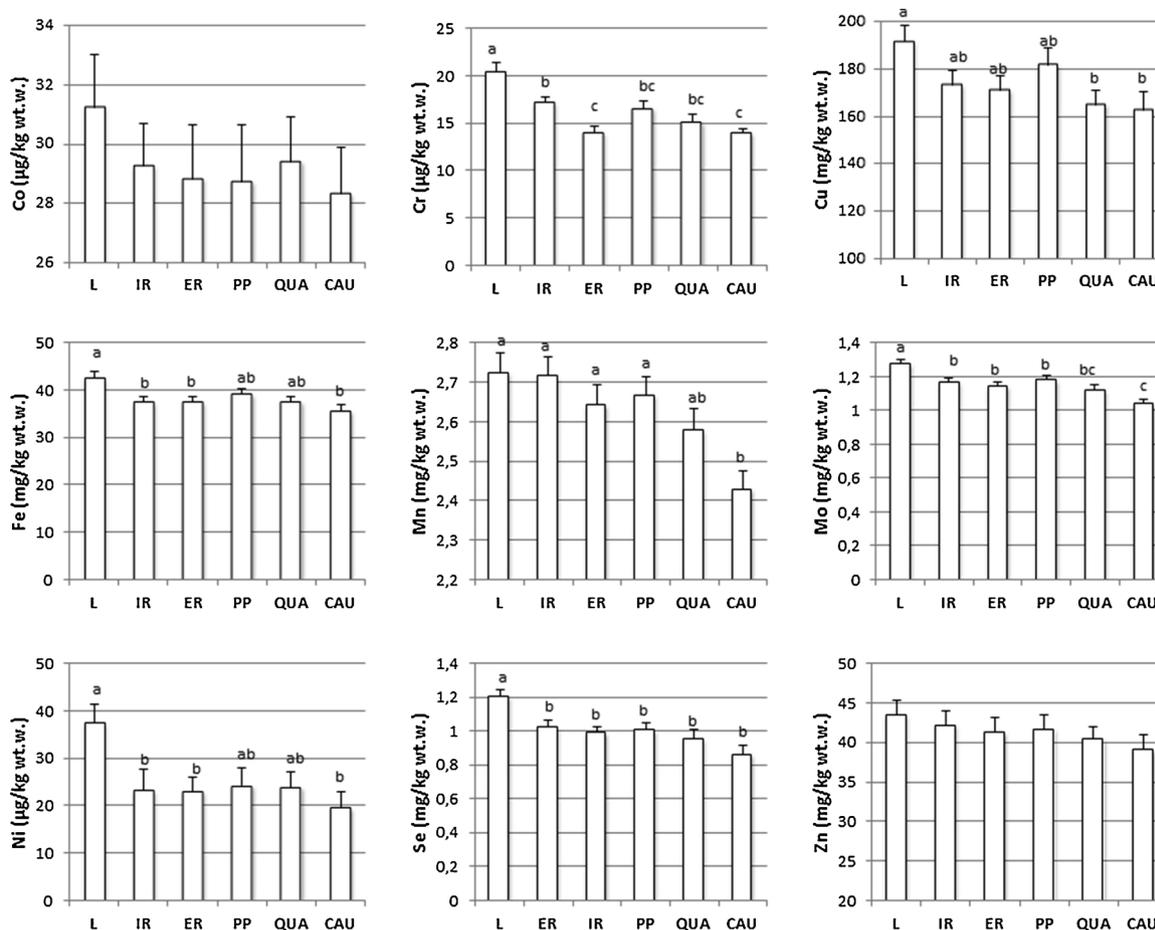
Fig. 1. Trace element concentrations in the liver lobes (L: left lobe, IR: internal right lobe, ER: external right lobe, PP: processus papilaris, QUA: quadrate lobe, CAU: caudate lobe). Different letters indicate statistically significant differences between lobes at $P < 0.05$.

In an attempt to better understand the higher trace element deposition in the left lobe in cattle in our study, the proportion (in %) of trace element concentrations in the different hepatic lobes relative to the left lobe (Y axis) was plotted against trace element concentrations in the left lobe (X axis) (Fig. 2). Overall, points above/below 100% indicate higher/lower trace element concentrations (respectively) in each lobe relative to the left lobe. For most trace elements, a larger proportion of the cloud-points were above 100% in the calves that showed the lower trace element concentrations in the left lobe, but decreased gradually as trace element concentrations in the left lobe increased. For those trace elements supplied at higher concentrations in the mineral supplemented concentrate feed relative to the physiological needs (copper, manganese and zinc) most of the cloud-point was below 100% for the range of concentrations in the left lobe. These results suggest again that the storage capacity of the left lobe is higher and/or starts sooner than the rest of the liver areas, making the left lobe the less appropriate sample as biomarker of the trace element status. On the contrary, the higher storage capacity of the left lobe would be interesting to biomonitor if excessive trace element accumulation in the liver can have negative consequences for the animal health or the consumer (e.g. to monitor excessive copper accumulation in cattle). Fig. 2 also enables evaluation of the variability in trace element deposition within the liver relative to the left lobe. This was generally below 50% (most samples being between 75–125% of those in the L lobe) for most of the trace elements (present at mg/kg wet weight (wt.w.) in the liver) except selenium. For those elements present at low concentrations (μg/kg wt.w.), the level of variability was higher (up to 300% for nickel), with a tendency towards higher concentrations in the left lobe for higher levels of trace element deposition.

The correlations between trace element concentrations within the different hepatic lobes are shown in Table 2. For the main trace elements, the correlations were generally very high and similar between all lobes, except CAU (Cu > 0.915; Zn > 0.906; Mn > 0.816; Mo > 0.675; Fe > 0.617 at $P < 0.001$ in all cases). These results suggest again a higher metabolic activity of the caudate lobe to export trace elements to tissues (i.e. caeruploplasmin or ferritin) and/or to biliary excretion. Again, the only exception was selenium, for which there were no significant associations between lobes; the reasons behind the different distribution of selenium in the liver compared to the other main trace elements are not known and would deserve further research. For the trace elements present at low concentrations (cobalt, chromium and nickel) the between-lobe correlations were very weak ($P < 0.01$). Altogether, these findings indicate that the pattern of distribution of the main essential trace elements (except selenium) across the liver, except in CAU, was very similar in the calves under study, and consequently the analysis of a single sample from these liver lobes would therefore be representative of the whole organ.

4. Conclusions

A single sample of liver from the same anatomical region (excluding the caudate and left lobes) would be adequate for determining the trace element status of cattle. This proposal is based on the main study findings, in which: (1) trace elements were unevenly distributed across the liver; (2) the left lobe appeared to have a higher capacity for trace element storage (which could be more important at high levels of exposure); and (3) the concentrations of the main trace elements were highly correlated across the liver (except for the caudate lobe and for
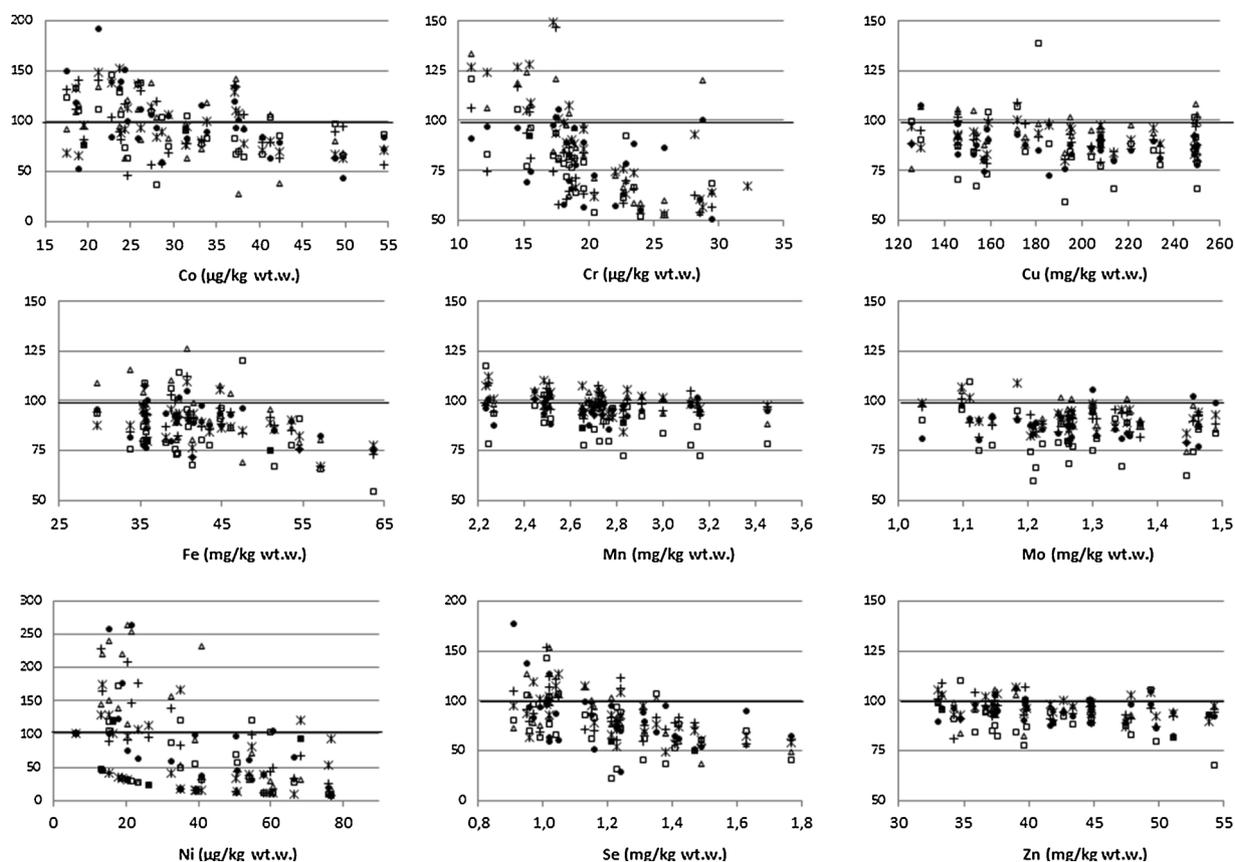
Fig. 2. Scatter plot showing the proportion (in %) of trace element concentrations in the different hepatic lobes relative to the left lobe (Y axis) against trace element concentrations in the left lobe (X axis). (Δ: processus papilaris, □: caudate lobe, ●: quadrate lobe, +: external right lobe, *: internal right lobe).

Table 2
Pearson's correlations between hepatic lobes (PP: processus papilaris, CAU: caudate lobe, QUA: quadrate lobe, ER: external right lobe, IR: internal right lobe, L: left lobe).

	Co	Cr	Cu	Fe	Mn	Mo	Ni	Se	Zn
PP vs CAU	0.340	0.060	0.773***	0.422*	0.445*	0.642***	−0.009	0.267	0.921***
PP vs QUA	0.310	0.602***	0.925***	0.617***	0.834***	0.675***	0.061	.0274	0.970***
PP vs ER	0.618***	0.195	0.930***	0.790***	0.841***	0.869***	0.117	.0064	0.973***
PP vs IR	0.568**	−0.162	0.925***	0.814***	0.830***	0.763***	0.010	0.239	0.979***
PP vs L	0.492**	0.202	0.931***	0.671***	0.882***	0.758***	−0.224	−0.169	0.975***
CAU vs QUA	0.223	0.156	0.764***	0.630***	0.377*	0.479*	0.114	0.257	0.892***
CAU vs ER	0.333	0.482**	0.817***	0.608***	0.414*	0.672***	0.368*	0.420*	0.913***
CAU vs IR	0.466**	0.107	0.773***	0.597***	0.499**	0.676***	0.215	0.198	0.906***
CAU vs L	0.639***	0.127	0.725***	0.507**	0.388*	0.459*	0.347	−0.050	0.916***
QUA vs ER	0.353	0.032	0.897***	0.781***	0.875***	0.715***	0.119	−0.199	0.958***
QUA vs IR	0.568**	−0.120	0.915***	0.769***	0.821***	0.684***	0.202	−0.171	0.969***
QUA vs L	0.462*	0.369*	0.930***	0.814***	0.907***	0.746***	0.211	0.089	0.968***
ER vs IR	0.489**	0.206	0.921***	0.886***	0.816***	0.767***	0.126	0.284	0.971***
ER vs L	0.587**	0.125	0.919***	0.740***	0.851***	0.813***	−0.030	0.025	0.969***
IR vs L	0.569**	0.207	0.940***	0.828***	0.839***	0.734***	0.276	−0.002	0.978***

* p < 0.05, ** p < 0.01 and *** p < 0.001.

selenium). Samples of the left lobe could be also considered for evaluating the risk of accumulation of high hepatic levels of trace elements, e.g. the risk of chronic copper toxicity.

Conflict of interest

None.

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