

## **Influence of farm type (organic, conventional and intensive) on toxic metal accumulation in calves in NW Spain**

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**Abstract.** The aim of the present study was to determine how accumulation of toxic metals by beef-cattle in NW Spain varies between farms that have markedly different practices (including intensive, conventional and organic management) and to determine possible key factors affecting toxic metal assimilation by cattle. Soil, feed (forage and concentrate) and animal tissues (liver and kidney from 120 calves) were collected from nine farms across NW Spain and were analysed for metals by ICP-MS. Toxic metal concentrations in beef calves were generally low but did vary significantly between farms. There were no consistent patterns of difference in tissue metal concentrations between farms from different regions or between farms with different management practices. Variations in arsenic, cadmium and mercury concentrations in calf tissues were not significantly explained by soil or diet metal concentrations but were significantly and inversely related to the proportion of concentrate in the ration. Higher levels of metal residues in tissues were associated with consumption of low amounts of concentrate and relatively high levels of grazing. Higher toxic metal intake due to grazing is likely to be largely a result of soil ingestion.

**Key words:** arsenic, mercury, cadmium, lead, farming practices, calves, grazing

### **INTRODUCTION**

Human activities associated with industry (mining, smelting, metal refining) and agronomy (application of mineral fertilisers, sewage sludge, lead-containing chemicals, arsenical pesticides) can contaminate the environment with toxic metals and metalloids (Brouwere et al., 2004; Nordberg et al., 2007). Such inputs, together with natural geological sources, largely determine the environmental distribution of toxic metals on agricultural land and so affect subsequent assimilation of metals by agricultural livestock. However, the extent to which those inputs affect exposure of livestock will also vary with farming practice. On intensive farms, a significant proportion of the diet is composed of concentrates that can include fish and feather meal, a significant source

of methyl mercury (Plummer & Bartlett, 1975; Jorhem et al., 1991). Animals are largely or exclusively reared indoors and so are unlikely to be affected by contamination of local pastures. In contrast, animals reared on conventionally managed and organic farms are largely or exclusively fed on local forage (mostly through grazing on pasture and being fed locally cut hay) and are often maintained mostly out of doors.

The aim of the present study was to determine how accumulation of arsenic, cadmium, mercury and lead by beef-cattle in NW Spain varies among farms (including farms that have intensive, conventional and organic management practices) and to identify possible key factors affecting toxic metal exposure across all farm types in NW Spain.

## MATERIALS AND METHODS

Livestock practices are highly standardized in intensive beef-cattle farms in NW Spain. In this region, the conventional (which has been the traditional) form of production involves many small farms that rear cattle using on-site sources of feed derived from locally-grown crops. Although the feed consists mainly (50–90%) of mother's-milk and local forage (fresh pasture or hay), the remainder of the diet is made up of commercial concentrate. Calves can be reared in an indoor, outdoor or mixed (in- and outdoor) system. In the 1980s, calves began to be produced on specialised farms where imported or purchased concentrate was used to promote rapid growth and weight gain. Most (70–90%) of the feed ration consists of imported concentrate and the rest is locally produced or purchased forage; cattle are reared indoors. In recent years however, there has been a growth in the number of organic farms in the region. In most cases, these organic farms are the result of conventional farms adapting their practices to the more regulated methods required to achieve “organic” status (CEC, 1999).

Samples were obtained from cattle from farms in the districts of Baralla (B) (42°52', 7°23'), Montederramo (M) (42°16', 7°30') and Vilalba (V) (43°18', 7°40') in Galicia. Surrounding organic (O), conventional (C) and intensive (I) beef farms were randomly selected. All components of the diet were in accordance with the practices and legislation associated with each farming system (CEC, 1999; 2005), and use of any mercury or arsenic based veterinary drugs (fungicides, wormers) was noted in the farm records.

Liver and kidney samples were collected at slaughter, when calves were aged between seven and ten months. Samples were packed in plastic bags, immediately placed on ice, transported to the laboratory, and stored at -18°C until processed. Soil and feed, both concentrate and locally produced forage, were collected from representative fields on each farm.

Approximately 2-g sub-samples of liver and kidney were digested in 5 ml of concentrated nitric acid (Suprapur grade, Merck) and 2 ml of 30% wv<sup>-1</sup> hydrogen peroxide in a microwave digestion system (Milestone, Ethos Plus, Italy). Digested samples were transferred to polypropylene sample tubes and diluted to 25 ml with ultrapure water. Arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) concentrations were determined by ICP-MS (VGElemental PlasmaQuad SOption). An analytical quality control programme was applied throughout the study. For tissue samples, the limit of detection in the acid digest, calculated as three times the standard

deviation of the reagent blanks, were 1.0 (As), 0.1 (Cd), 0.2 (Hg) and 0.3 (Pb)  $\mu\text{g l}^{-1}$ . Analytical recoveries were determined from a certified reference material (Pig kidney CRM 186, BCR Reference Materials, Belgium) analysed alongside the samples. The mean  $\pm$  SD measured (certified values in parenthesis) concentrations ( $\mu\text{g kg}^{-1}$  dry weight) were  $69 \pm 6$  ( $63 \pm 9$ ) for As,  $2711 \pm 122$  ( $2710 \pm 150$ ) for Cd,  $1852 \pm 111$  ( $1970 \pm 40$ ) for Hg and  $318 \pm 41$  ( $306 \pm 11$ ) for Pb. The precision of the analytical method, calculated as the relative standard deviation of toxic metal concentrations in 10 digests of the same sample were 8.4% (As), 4.4% (Cd), 3.2% (Hg) and 10.2% (Pb). For soil, feed samples, limits of detection in the acid digest were 1.0 (As), 0.3 (Cd), 1.1 (Hg) and 0.4 (Pb)  $\mu\text{g l}^{-1}$  and 1.1 (As), 0.15 (Cd), 1.0 (Hg) and 2.4 (Pb)  $\mu\text{g l}^{-1}$  respectively. Analytical recovery, determined from certified reference materials (Forest Soil ISE 985; Barley IPE 548; Grass ISE 686; Wepal Reference Materials, Wageningen University) were between 76% and 123%.

All statistical analyses were made using the program SPSS for Windows (v.15.0). To calculate mean metal concentrations in the different tissues, non-detectable concentrations were assigned a value of half quantification limit. Normal distribution of data was checked using a Kolmogorov-Smirnov test. Data for all liver and kidney concentrations were not normally distributed and were log-transformed before analysis, so average liver and kidney As, Cd, Hg and Pb concentrations are given as geometric means. Analysis of variance and post-hoc Tukey's honest significant difference (HSD) tests were used to evaluate variation in tissue metal concentrations among farms. Differences in tissue metal concentrations between the liver and the kidney were assessed using a paired t-test and intra-tissue toxic metal correlations were calculated by Spearman rank correlations. Backwards stepwise multiple regression was used to analyse the relationship between animal tissue and soil and feed toxic metal concentration. In all analyses statistical significance was taken to be indicated by  $p < 0.05$ .

## RESULTS

Arsenic concentrations in the tissues of calves in this study varied between non-detected and  $103 \mu\text{g kg}^{-1}$  wet weight. Arsenic concentrations were significantly higher in the liver than the kidney ( $t_{(117)}=9.178$ ,  $P=0.000$ ,  $n=118$ ) and tissue arsenic concentrations varied significantly among farms, although there was more variability in arsenic concentrations among farms for kidney than for liver. Overall, arsenic residues tended to be higher in calves from Montederramo compared to animals from other regions for both the liver (23–29% higher) and the kidney (58–60% higher). There was no obvious pattern of differences in tissue arsenic accumulation among farms with different farming practice, and although intra-farm variability in arsenic liver concentrations tended to be lowest for intensive farms, this was not true for kidney arsenic. The ratio of liver:kidney arsenic accumulation was significantly higher ( $F_{2,117}=4.437$ ,  $P=0.014$ ) in intensive (3.29) than organic (2.00) and conventional (2.11) calves.

Cadmium concentrations in calf liver and kidney tissues ranged between non-detected and  $189 \mu\text{g kg}^{-1}$  wet weight, and were significantly higher (up 2-fold) in the kidney than in the liver ( $t_{(117)}=-10.051$ ,  $P=0.000$ ,  $n=118$ ). With the exception of the liver cadmium concentration in animals from Montederramo, calves from organic farms had

the highest cadmium residues found in farms from the same region. The difference in kidney cadmium concentrations between calves from organic and intensive farms was particularly marked (41–64% higher). Kidney Cd concentrations appeared to be most variable in calves from conventional farms. The ratio of liver:kidney cadmium accumulation was significantly higher ( $F_{2,117}= 31.076$ ,  $P=0.000$ ) in intensive (0.715) than organic (0.376) and conventional (0.486) calves.

A large proportion (78%) of calf livers did not contain detectable amounts of mercury and concentrations in most of the livers that had detectable levels were close to the quantification limit (1–2  $\mu\text{gkg}^{-1}$ ). Mercury accumulation in calves was significantly greater in the kidney ( $t_{(117)}=-10.074$ ,  $P=0.000$   $n=118$ ) and renal mercury residues tended to be higher in calves from farms in the Vilalba region (12–36% higher than mean concentrations in calves from farms in other regions), although there were no consistent significant differences among regions for farms with the same management practice.

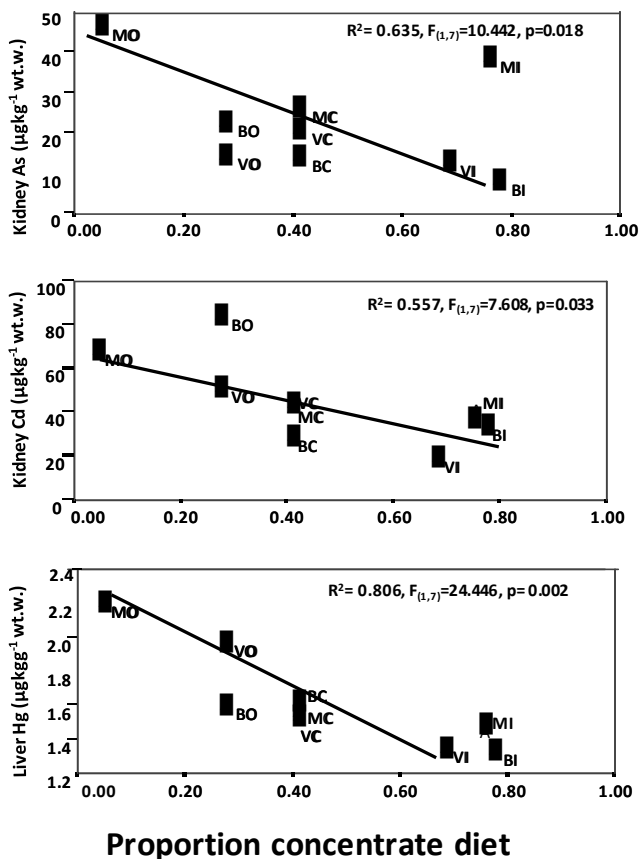
Lead concentrations in calves in this study ranged from 4.11 to 220  $\mu\text{gkg}^{-1}$  wet weight and concentrations were on average 2-fold higher in the kidney than the liver ( $t_{(117)}=-6.850$ ,  $P=0.000$   $n=118$ ). Higher (2-fold) lead residues were detected in calves from Montederramo compared with animals from other regions but there were no consistent differences in tissue lead concentrations that could be related to farm management practice.

When we study correlations between toxic metal concentrations in tissues in calves in our study, positive associations were found between arsenic and lead both in the liver and kidney, and between arsenic and cadmium in the kidney.

In relation to toxic metal concentrations in soils from the different farms, mercury concentrations were below the detection limit in all samples. For the other elements, soil concentrations were similar for the three farm types in each district, although, in general, there was a tendency for metal concentrations in soils on organic farms to be lower than those on conventional and intensive farms. The mean soil arsenic concentrations in farms from Vilalba were a third of those for farms from other districts.

In relation to toxic metal concentrations in feed, Hg concentrations were below the detection limit in all the forage samples. For the other toxic metals, concentrations in locally produced forage tended to be more variable than concentrations in soil. There was no obvious pattern in forage metal residues with region or farm management practice, although toxic metal concentration in concentrates on two of the organic farms (in Montederramo and Vilalba) had the lowest toxic metal residues measured in concentrates in this study; toxic metal levels tended to be similar for concentrates used on conventional and intensive farms. In general, and with the exception of organic farms in Montederramo and Vilalba, arsenic and cadmium residues tended to be higher in concentrates than in local forage, whereas lead residues tended to be higher in local forage.

The backwards stepwise multiple regression model showed that neither soil nor concentrate toxic metal concentrations were significant parameters ( $P>0.1$  in all cases) in the models (Fig. 1).



**Fig. 1.** Scatterplot showing the relationship between toxic element residues in calves ( $\mu\text{g}/\text{kg}$  wet weight) and the proportion of concentrate in the diet in the farms in our study. Abbreviations for farms are as follows B: Baralla, M: Montederramo, V: Vilalba, C: Conventional, I: Intensive, O: Organic.

The proportion of concentrate in the ration was inversely related to kidney arsenic and cadmium concentrations and was a significant factor in the statistical models, explaining 63 and 55% of the variability respectively (Fig. 1). The proportion of concentrate in the diet was similarly associated with liver arsenic and cadmium residues but was not a statistically significant factor. The inclusion of arsenic concentration in forage slightly improved the overall fit of the model explaining variation in kidney arsenic but it was not a significant term in the analysis. The proportion of concentrate in the ration was also important in explaining mercury assimilation. There was an inverse relationship between proportion of concentrate in the diet and liver mercury and the proportion of concentrate accounted for 80% of the variability in hepatic mercury residues among calves from different farms. There was likewise a significant negative relationship ( $R^2 = 0.822$ ,  $F_{1,7} = 32.3$ ,  $P = 0.001$ ) between the proportion of concentrate in the ration and the proportion of calves on the farm with detectable liver mercury concentrations.

## DISCUSSION

Differences in toxic metal concentrations in the diet (forage and concentrate) did not explain the variation among farms. Organically reared calves generally had higher tissue cadmium residues than animals from conventional and intensive systems in the same area, despite having similar or lower cadmium levels in the diet.

Renal arsenic and cadmium and hepatic mercury accumulation in calves were inversely related to the proportion of concentrate in the ration. Higher toxic metal accumulation in grazing calves could be related to soil ingestion.

The liver:kidney ratio for arsenic and cadmium was greater in intensive calves. Differences in toxic metal accumulation among production systems previously described in adult cattle (López-Alonso et al., 2003) and were related to the higher metabolic activity and blood-flow through the liver in intensively reared calves fed with an energy-rich diet.

The lower intra-farm variation tended to be for intensive farms. This is likely to be due to the high level of standardization of the diet and in annual husbandry practices, and soil ingestion (metal exposure) being lower due to the small proportion of the forage in total diet. In contrast, the highest variation occurred in conventional farms. These farms have less standardized husbandry practices and the extent of grazing (and associated soil ingestion) can vary between animals and during the year because of pasture conditions.

## CONCLUSIONS

Toxic metal accumulation in cattle in our study was highly dependent on the proportion of concentrate in the ration (an index of the extent to which animals grazed). Higher levels of toxic metal residues were found in calves that obtained more nutrition through grazing (low proportion of concentrate in the diet), and this may well be associated, at least in part, to soil ingestion when grazing.

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