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Cadmium accumulation in liver and kidney of sheep grazing ryegrass/white clover pastures

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ABSTRACT

Pastures growing on soils fertilised with phosphatic fertilisers accumulate cadmium (Cd) in their plant tops in proportion to substrate concentrations. Sheep grazing pastures absorb some of this Cd. To quantify the rate with which Cd is accumulated in sheep tissue, three replicated 0.4 ha blocks were fertilised with either 333 kg/ha of single superphosphate only (control) or amended with $CdSO_4$ to give 274g Cd/ha (high-Cd), and set stocked with 10, three months old Romney wether lambs. Cadmium (Cd) concentrations were determined in liver, kidney, proximal duodenal and skeletal muscle tissue of sheep, ingesting either (means ± s.e.) 266±22 (control) or 746±58 (high-Cd) µg Cd/day from three to six months or 290±21 and 721±72 µg Cd/day over six to eighteen months. Cadmium concentrations in kidney and liver tissue increased markedly over the first three months: 0.042±0.006 to 0.32±0.03 (Control) and 0.57±0.04 (high-Cd) µg Cd/g fresh tissue, and 0.04±0.006 to 0.12±0.02 (Control) and 0.31±0.06 (high-Cd) µg Cd/g fresh tissue, for kidney and liver respectively. Although total Cd content continued to increase, as organ size increased, over the next 12 months daily accumulation rates in both tissues were much lower compared with those over the first three months. For example, daily Cd accumulation in kidney decreased from 0.25±0.002 to 0.06±0.01 and 0.47±0.04 to 0.32±0.05 (µgCd/d) for sheep from the control and high-Cd pastures respectively. Total Cd content in kidney and liver combined accounted for approximately 0.25% of total Cd ingested by sheep age three to six months but this significantly decreased to 0.1% in sheep age six to eighteen months.

At higher Cd intakes increased metallothionein protein synthesis in kidney and liver tissue enabled animals to be more efficient in sequestering Cd. This resulted in reduced accumulation of Cd in muscle tissue of sheep on the high-Cd pastures (2.0±0.04 and 5.0±0.08 ng Cd/g) compared with those grazing the control pasture (4.0±0.6 and 14±5 ng Cd/g) in animals from the first and second periods respectively.

The results from this study show that it is the first few months after weaning that fastest uptake into kidney occurs and therefore strategies to reduce Cd absorption and subsequent accumulation, e.g. by increasing zinc in the diet, should focus on young animals.

Keywords: Cadmium; zinc; accumulation; sheep tissues; metallothionein.

INTRODUCTION

Past and present fertiliser usage has resulted in an accumulation of cadmium (Cd) in soils and elevated Cd concentrations in plants (Bramley, 1990). Sheep and other ruminants grazing pastures absorb a small but significant proportion of this Cd, some of which is subsequently stored in liver and kidney. Because Cd turnover in the kidneys is very slow, (Fox, 1986) the Cd content of kidneys increases with age of the animal. A substantial proportion of kidneys from sheep are rejected for human consumption because it is likely that Cd concentrations will exceed 1 mg/kg fresh tissue (the NZ maximum residue level for Cd). Because of the uncertainty in the content of Cd in kidneys, of older sheep in particular, and the impracticality of analysing kidney tissue from every sheep, present regulations call for the exclusion for export of kidneys from sheep over 30 months of age. Surveys monitoring the Cd concentration in sheep kidney tissue both in NZ (MAF Regulatory Authority) and in Australia (Petterson et al., 1991) show that concentration increases with age of the animal. However the Australian data, which gave a linear relationship between the logarithm of the concentration of Cd and age, showed considerable within-site and between-site variation in the rate of accumulation. Recent NZ survey data (Dr B. Marshall, *pers. comm.*) showed that even in young sheep (< 30 months) the data was heavily skewed with more than 22% of kidney's greater than 1mg Cd/kg fresh tissue.

Although the problem of Cd residues in offal is by no means confined to N.Z. alone, there is concern that the presence of even moderate amounts of Cd in NZ offal exports could be used as an indirect tariff barrier. Currently the loss of export earnings is relatively minor, but this may increase should the Cd residue limit in either kidney or liver tissue be reduced. Furthermore, publicity associated with the presence of Cd in meat products is detrimental to the current image NZ has as a "safe food producer".

Cadmium in tissues is bound mainly to metallothionein (MT), a low molecular weight protein present in sub-cellular fractions. Synthesis of this protein is induced by Cd and this provides an important mechanism for detoxification by protective sequestering of the metal (Friburg, *et al*, 1986). Because MT is also involved in Zn and Cu regulation, many of the observed interactions between Cd and Zn and Cu may be mediated through changed MT expression (Bremner, 1987). Further, homeostatic mechanisms to limit Cd absorption are poor (Fox, 1988) and sheep grazing elevated Cd containing pastures are therefore likely to accumulate correspondingly greater amounts of the metal in kidney and liver tissue. This paper reports on preliminary data establishing the relationship between Cd concentrations in pastures and it's rate of accumulation in tissues from sheep grazing New Zealand pastures from three to eighteen months of age and is part of a larger investigation to quantify Cd metabolism in sheep. Because of possible interactions between Cd and Zn, the latter element was also determined in various tissues.

MATERIALS AND METHODS

Experimental design

In December 1991, three replicated paired 0.4 ha blocks on medium slope hill country were fertilised with a single application of either 333 kg/ha of single superphosphate or superphosphate amended with $CdSO_4$ to give 274 g Cd/ha. This input of Cd, based on preliminary trials, elevated Cd pasture concentrations about three fold over background (0.15-0.2 μg Cd/g DM) for an eighteen month period. Each block was set stocked with 10 Romney wether lambs three weeks after fertilisation. Pasture samples were collected monthly from under specially designed Cd-free plastic frames and the Cd concentration determined in a mixed, unwashed pasture sub-sample.

Daily feed DM intakes of individual sheep were determined with intraruminal chromium sesquioxide controlled release capsules administered three weeks prior to slaughter and measuring chromium in faecal material collected over a two week period (Parker *et al.* 1990). This data and Cd concentrations in the monthly pasture samples enabled the mean daily Cd intake to be estimated over the experimental period.

Prior to the start of the experiment a pretreatment group of 10 lambs (aged three months) were slaughtered. Groups of 10 sheep grazing the control (0.15-0.2 μg Cd/g DM) and treatment (0.4-0.6 μg Cd/g DM) Cd containing pastures were subsequently slaughtered after three and fifteen months grazing (sheep aged approximately six and eighteen months respectively). Liver, kidney, duodenum and a sample of skeletal muscle (hind leg) were removed, and major organs weighed.

Sample preparation and analysis

Collection of tissues, sample preparation, Zn analysis and quantification of metallothionein-mRNA has been previously described (Lee *et al*, 1994). Briefly tissues were washed in physiological saline, subsampled and then frozen in liquid nitrogen prior to homogenisation and freeze-drying. Latex gloves and surgical-grade stainless steel instruments were used to handle tissues to minimize Cd contamination. Cadmium was determined by Zeeman electrothermal atomic absorption (Varian Spectra AA-400) (Grace *et al*, 1993). Chromium sesquioxide was analysed in subsamples of pooled faecal material using a sodium peroxide fusion digestion method and plasma emission spectrometry (Lee *et al*, 1986).

RESULTS

Concentrations of Cd in kidney and liver tissue at each slaughter period for animals grazing control (0.1-0.2 μg Cd/g DM) and treatment (0.4-0.6 μg Cd/g DM) Cd containing pastures are shown in Table 1. Cadmium concentration in both kidney and liver tissue increased markedly over the first three months (animals aged three to six months). Over the next twelve month period there was little change in either kidney or liver Cd concentrations, apart from that in kidneys of animals grazing the high Cd pasture (0.57 to 1.11 μg Cd/g tissue). However as the mean fresh weight for a single kidney increases with age (45 g at six months to 72 g at eighteen months of age), total Cd content continues to increase (Table 1). Similarly total Cd content in liver also increases. Animals grazing the high Cd pasture had significantly greater Cd concentrations in both liver and kidney tissue than those grazing normal pasture.

TABLE 1: Mean (± standard error) concentrations of Cd in fresh kidney and liver tissue from sheep grazing control (0.1-0.2 μg Cd/g DM) and high (0.4-0.6 μg Cd/g DM) Cd containing pastures (n=10).

	Age (months)	Cd concentration (μg Cd/g tissue) Control	High	Effect[1]
Kidney	3	0.042±.006 (2)[2]		
	6	0.32±0.03 (15)	0.57±0.04 (25)	P<0.001
	18	0.33±0.05 (24)	1.11±0.20 (75)	P<0.002
Liver	3	0.04±0.006	—	
	6	0.12±0.02	0.31±0.06	P<0.005
	18	0.12±0.008	0.41±0.04	P<0.001

[1]Significance of difference between means.
[2]Total Cd content (μg) in one kidney.

Table 2 gives data for Cd in hind leg muscle and duodenal tissue from sheep grazing control and treated pastures which were slaughtered at six and eighteen months of age. Cadmium concentrations in muscle from sheep grazing the high Cd pasture were lower (significantly at six months of age) than that in muscle of sheep from the control Cd pastures, although in both treatment groups Cd muscle concentrations increased with age. In contrast Cd concentrations in duodenal tissue were higher in sheep fed high Cd pasture, but concentrations were unchanged or decreased with age.

TABLE 2: Mean (± standard error) concentrations of Cd (ng Cd/g) in fresh muscle and proximal duodenal tissue from sheep grazing control (0.1-0.2 μg Cd/g DM) and high (0.4-0.6 μg Cd/g DM) Cd containing pastures (n=10).

	Age (month)	Control	High	Effect[1]
Muscle	3	2±0.9	-	-
	6	4±0.6	2±0.04	P<0.005
	18	14±5	5±0.08	NS
Duodenal	3	15±14	-	-
	6	11±2.5	50±7	P<0.001
	18	10±1.5	30±2.3	P<0.001

[1]Significance of difference between means.

Mean daily accumulation rates for Cd in the two kidneys and liver of sheep ingesting Cd at the two levels of intake over two periods of time are shown in Table 3. Data has been derived from the Cd concentration data (Table 1) and organ weight. There was no significant difference in organ weights between the two treatment groups. Pasture intakes (means ± se) were 1027±50 and 1566±136 g DM/day for the first (3-6 months) and second (6-18 months) time periods respectively. The concentration of Cd in pasture slowly declined over the experimental period from 0.27±0.04 to 0.18±0.02 µg Cd/g in control herbage and from 0.69±0.06 to 0.49±0.08 µg Cd/g in the high Cd herbage. Hence the increased intakes of older lambs, with reduced Cd concentrations in herbage, resulted in a similar intake of Cd over the entire experimental period (Table 3).

TABLE 3: Mean (± standard error) daily accumulation rate of Cd by the kidneys and liver organs and daily Cd intake by sheep grazing control (0.1-0.2 µg Cd/g DM) and high (0.4-0.6 µg Cd/g DM) Cd containing pastures.

Period (months)	Treatment	Intake (µg Cd/d)	Cd accumulation (µg Cd/d) Kidney	Liver
3-6	Control	266±22	0.25±0.002	0.41±0.06
	High	746±58	0.47±0.04	1.32±0.22
6-18	Control	290±21	0.06±0.01	0.14±0.07
	High	721±72	0.32±0.05	0.54±0.06

As expected, mean daily total Cd accumulation in kidney and liver was greater in sheep grazing the high Cd pasture. In terms of total Cd content the liver accumulated more than the kidneys, while both liver and kidney combined accounted for approximately 0.25% of total Cd ingested over the three to six month period. This decreased ($P<0.001$) to about 0.1% of intake over the six to eighteen month period. The relative decrease with time in daily accumulation rate was greater for animals grazing the control Cd pastures than for those on the high Cd pastures, for example 0.25 to 0.06 µg Cd/day in kidneys from the control group compared with 0.47 to 0.32 µg Cd/day in kidneys from the high Cd group.

Zinc and MT mRNA were determined in kidney tissue because of the possible effect of Cd on Zn metabolism and the role of MT on Cd storage in the kidney. Data for sheep aged 6 months grazing the control and high Cd pastures are given in Table 4. Increased Cd intakes, concomitant with increased Cd concentrations in kidney tissue, significantly increased both tissue Zn concentrations and MT mRNA expression, with MT-II mRNA in kidney tissue from sheep in the high Cd group about twice that of the control group. This matches the observed increase in Cd concentration for the same tissue (Table 4).

TABLE 4: Influence of Cd intake on mean (± standard error) Zn concentrations and metallothionein-II mRNA expression in kidneys from sheep aged 6 months grazing control (0.1-0.2 µg Cd/g DM) and high (0.4-0.6 µg Cd/g DM) Cd containing pastures.

	Control	High	Significance of effect
Cd (µg/g fresh tissue)	0.32±0.03	0.57±.04	P<0.001
Zn (µg/g fresh tissue)	28±0.8	32±0.8	P<0.001
MT-II mRNA (molecules/pg Total RNA)	11±2	20±3	P<0.02

DISCUSSION

Although total Cd in sheep kidney, and to a lessor extent in liver, continues to increase with age, the rate of increase rather than being linear with time as might be expected, decreases with age of the animal. It is the first few months after weaning that the fastest uptake of Cd into kidney occurs. These observations are supported by recent survey data of Cd concentrations in kidneys collected from abattoirs which indicate that even in younger animals (30 months) a significant proportion still exceed the 1 mg Cd/kg maximum residue level (Dr B Marshall, *pers. comm.*). Therefore strategies to minimise Cd accumulation in sheep kidney must focus on young animals.

There is little homeostatic control to limit Cd absorption, thus as Cd intake increases (at least initially) so does tissue retention. This has been previously demonstrated in a number of other animal species (Fox, 1988). We have shown that over a longer period of time the animal responds to the increase in absorbed Cd and adapts its metabolism of this element in such a way as to minimise both absorption and retention. This occurs in sheep grazing NZ pastures which have lower than average Cd concentrations (0.15-0.2 µg Cd/g DM) as well as in those having high concentrations (0.4-0.6 µg Cd/g DM). At the higher Cd intakes increased MT protein synthesis (Table 4) enables the animal to be even more efficient in sequestering Cd, with subsequent storage in kidney and/or recycling back into the gut via various bile and mucous secretions (Grace *et al.* 1993). This results in reduced Cd concentrations in muscle tissue compared with sheep having lower Cd intakes (Table 2). Further evidence for reduced absorption over time may be indicated by the lower concentrations of Cd in duodenal tissue of sheep from the high Cd intake group at 18 months of age compared with those aged 6 months.

Although daily net retention of Cd in kidney and liver was only a small percentage of total intake (about 0.25% up to six months of age decreasing to 0.1%at age 18 months), the 75 µg total accumulated Cd in one kidney (when the tissue concentration is about 1 µg Cd/g fresh tissue) approximates the daily tolerable intake for humans (FAO/WHO, 1989). Total body Cd in these sheep was about 1% of daily intake (authors, unpublished data). Thus, although these accumulation rates are small there is considerable demand to prevent or at least modify current accumulation rates. A reduction in Cd fertiliser content is obvious, although this would not alter existing soil Cd levels in the short term, as Cd is relatively immobile in the upper soil profile (Williams and David, 1976), and the majority of Cd ingested by ruminants is returned, via the faeces, to the pastoral system. Our current studies indicate that predisposition for accumulation, the balance between excretion and retention of absorbed Cd, may vary according to the MT status of the animal. Cadmium is accumulated in kidney mostly in association with MT and this protein is also involved with absorption and excretion of the metal. Metallothionein is also induced by Zn and, as Cd and Zn have been shown to be mutually antagonistic (Hipp *et al.* 1991), increasing the Zn intake of sheep may be an effective means of controlling and therefore reducing the absorption and/or accumulation of Cd in tissues. This aspect of Cd metabolism is currently being investigated.

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