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## Intake and excretion of cadmium in sheep fed fresh herbage

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### ABSTRACT

Nine Romney sheep fed fresh herbage were used to study cadmium (Cd) metabolism. The major route for the excretion of ingested Cd is the faeces with less than 0.5 percent being lost in the urine. Although there was a marked net secretion of Cd into the digestive tract post duodenally a considerable net absorption occurred from the intestinal region. Absorbed Cd is mainly accumulated in the liver and kidney (>340 ng/g fresh tissue) with much small amounts being associated with muscle (<20 ng/g fresh tissue). As the variation in the intake and excretion of Cd is large, short term balance studies are less suitable as an approach to determine the rate of apparent retention of Cd in grazing animals. The use of long term slaughter techniques and kinetic studies using stable isotopes of Cd are discussed as the preferred methods to measure the absorption and retention of Cd.

**Keywords:** Cadmium, tissue accumulation, kidney, liver, sheep, fresh herbage.

### INTRODUCTION

A characteristic feature of cadmium (Cd) metabolism in animals is its poor homeostatic control and accumulation in the kidneys and liver (Mills and Dalgarno, 1972). This may pose a potential problem, as these edible offals cannot be exported when their Cd concentration exceeds 1 mg Cd/kg fresh tissue. The Cd concentration in NZ soils and pastures has gradually increased as a result of the regular and extended usage of phosphatic fertilisers which contained varying trace amounts of Cd depending on their source (Syers *et al.*, 1986).

Cadmium is a non essential metal which because of its toxic properties (Friberg, *et al.*, 1988) has been widely studied in small animals and humans. However the few studies reported for ruminants are based on semi synthetic diets with Cd contents very much higher than those encountered by grazing animals in NZ (Mills and Dalgarno, 1972). This study is part of a larger investigation on the Cd metabolism of sheep and reports on the intakes, distribution in the digesta and site of absorption, retention and excretion of Cd in sheep fed fresh herbage.

### MATERIALS AND METHODS

#### Animals and diets

Nine 8 month old castrated Romney sheep mean liveweight 30 kg, were divided into 3 groups and placed in wooden metabolism crates with stainless steel grated floors specially designed for trace metal studies. The sheep were fed mixed pasture (20% clover, 80% ryegrass) or *Lotus corniculatus* or *Lotus pedunculatus* respectively for 22 days. The three pastures grown in the same area, were cut daily and after determining the DM in a microwave oven a daily total of 800g DM was placed on a belt feeder and fed as hourly meals. About 4 weeks prior to the start of the study all sheep were fitted with a 40 mm rumen fistulae and a 10 mm T-shaped cannulae in the abomasum close to the pylorus.

#### Experimental design

After 12 days on the various diets a solution containing 0.005M Cr-EDTA and 0.2  $\mu$ Ci  $^{103}$ Ru-(1,10)-phenanthroline was infused at the rate of 450 to 690 ml/day into the rumen to measure the flow of digesta (liquid and solid phases respectively) entering the small intestine (Faichney, 1975; Tan *et al.* 1971). From day 14 the diet, feed residues, faeces and urine were collected daily to determine the intake and excretion of Cd. All samples were collected in polyethylene containers using procedures designed to minimise Cd contamination. From day 19, samples of rumen contents and abomasal digesta were collected in polyethylene containers according to the methods described elsewhere (Joblin and Lee 1990).

Animals were slaughtered on the 22nd day and the liver, kidney, heart, lung, pancreas, duodenum and a sample of skeletal muscle and skin removed.

#### Sample preparation and analysis

Samples of diet, feed residues, faeces, digesta and urine were collected daily and immediately frozen. Subsamples, except the urine, were then freeze dried, ground and 400 mg samples were wet ashed using 'Aristar' nitric acid. The digestion residue was then redissolved in 0.16M nitric acid and Cd determined by Zeeman electrothermal atomic absorption spectroscopy (ZAAS) according to standard procedures (Rothery 1986).

The tissues from the slaughtered sheep were washed in physiological saline, subsampled and then frozen in liquid nitrogen prior to homogenisation and freeze drying. To minimise contamination gloves and stainless steel instruments were used to handle the tissues. Blood samples (50 ml) were collected by veni-puncture into polyethylene tubes containing 100 i.u. heparin and centrifuged at 2000g for 20 minutes to separate plasma and red blood cells which were then stored at -10°C. Tissues were digested and analysed for Cd as described for the diet. Samples of plasma (10g) and

**TABLE 1:** Mean ( $\pm$  standard error) concentrations and quantities of Cd associated with the diet, abomasal digesta, faeces and urine of sheep fed three fresh herbage.

	Pasture		<i>Lotus corniculatus</i>		<i>Lotus pedunculatus</i>	
	Cd content ng/g DM <sup>1</sup>	Cd flow $\mu$ g/d	Cd content ng/g DM	Cd flow $\mu$ g/d	Cd content ng/g DM	Cd flow $\mu$ g/d
In diet	55	34.3 $\pm$ 2.85	112	71.1 $\pm$ 3.61	108	58.3 $\pm$ 3.45
At abomasum	172.5	56.5 $\pm$ 4.70	309.3	107.2 $\pm$ 9.43	507.6	126.9 $\pm$ 45.2
In faeces	231.0	37.5 $\pm$ 5.35	375.3	57.1 $\pm$ 11.86	667.6	63.0 $\pm$ 3.50
In urine	0.063	0.17 $\pm$ 0.01	0.055	0.11 $\pm$ 0.03	0.04	0.10 $\pm$ 0.02

<sup>1</sup>except urine which is  $\mu$ g/ml.

urine (50g) were digested in 'aristar' nitric acid, the residue redissolved in 0.016M nitric acid and adjusted to pH 6.0 with 'aristar' ammonium hydroxide before washing through a 1 cm<sup>3</sup> column containing 8-hydroxyquinoline glass-pore resin to preconcentrate the Cd and remove interfering matrix elements (Na, P) (Sturgeon *et al.*, 1981). The resin was eluted with 1M HNO<sub>3</sub>-0.1M HCl and the Cd in the eluent determined by ZAAS. The rumen and abomasal digesta were centrifuged according to the methods of Joblin and Lee (1990) and the pellet (solid phase) and the supernatant (liquid phase) analysed for Cd as described above for the diet and plasma respectively. Chromium was measured in the digesta supernatants by plasma emission spectrometry (Joblin and Lee, 1990) and <sup>103</sup>Ru by gamma radiation counting (Tan *et al.*, 1971). The flow of Cd into the small intestines was from the determined daily flows of Cr and <sup>103</sup>Ru in the digesta as outlined by Faichney (1975) and the concentration of Cd in the digesta.

## RESULTS

The mean ( $\pm$  standard error) daily DM intakes for the sheep fed mixed pasture, *Lotus corniculatus* and *Lotus pedunculatus* were 629 $\pm$ 14, 635 $\pm$ 15 and 555 $\pm$ 16 respectively. The mean concentrations of Cd associated with the diet, faeces and urine for the sheep fed the various diets are given in Table 1. Also given in Table 1 are the daily quantities of Cd entering the small intestine (i.e. leaving the abomasum) calculated from the product of the combined solid and liquid flows and the Cd concentration in the respective phases.

Pooling the data over all diets the relationships between Cd intakes (X) and the Cd entering the small intestine (Y<sub>1</sub>) and excreted in the faeces (Y<sub>2</sub>) are described by the following regression equations:

$$Y_1 = -11.9 \pm 42.93 + (1.93 \pm 0.726)X; \quad r = 0.76, \text{ df}=6$$

$$Y_2 = 7.8 \pm 6.77 + (0.85 \pm 0.118)X; \quad r = 0.72, \text{ df}=6$$

From the above equations it can be calculated that at a Cd intake of 50  $\mu$ g/d the daily amounts of Cd entering the small intestine and excreted in the faeces are 84.6 and 50.3  $\mu$ g/day respectively. This gives a net secretion of 34.6  $\mu$ g Cd/day into the stomach region after which there is a net absorption of 34.3  $\mu$ g Cd/day from the intestinal region. Only about 0.5% of the Cd in the rumen contents (pH 5.8-6.5) was found to be associated with the liquid phase but this increased to over 30% in the acidic (pH 2.5-3.0) abomasal digesta.

The mean Cd concentrations in various organs and tissues of sheep fed various diets are given in Table 2.

**TABLE 2:** Mean ( $\pm$  standard error) Cd concentrations (ng/g DM) in organs and tissues of sheep fed three fresh herbage.

	Mixed pasture	<i>Lotus corniculatus</i>	<i>Lotus pedunculatus</i>
Liver	1159 $\pm$ 86	1491 $\pm$ 268	1621 $\pm$ 293
Kidney	339 $\pm$ 72	587 $\pm$ 137	639 $\pm$ 81
Pancreas	243 $\pm$ 35	300 $\pm$ 17	291 $\pm$ 38
Duodenum	190 $\pm$ 94	260 $\pm$ 50	321 $\pm$ 14
Lung	31 $\pm$ 0.5	25 $\pm$ 1.5	69 $\pm$ 24.7
Heart	41 $\pm$ 4.5	30 $\pm$ 1.2	45 $\pm$ 4.5
Skin	38 $\pm$ 4.5	29 $\pm$ 3.5	79 $\pm$ 27.1
Muscle	13 $\pm$ 2.0	6 $\pm$ 0.3	7 $\pm$ 1.0

The highest Cd concentrations were found in the liver followed by the kidney, pancreas and duodenum with the lowest concentration being associated with the skeletal muscle. However it must be pointed out that as part of the larger study 150 to 160  $\mu$ g of Cd-106, an amount larger than that which would be absorbed from the digestive tract, was infused intravenously over the last 5 days of the study (after the balance had been completed) and therefore some of the tissue Cd, particularly in the case of the liver, will not be of dietary origin.

## DISCUSSION

The differences in the Cd content of the 3 pasture species must reflect differences in their uptake and retention of Cd because all plants were grown in the same area, harvested in a similar fashion and there was little difference in the Cd concentrations of washed and unwashed samples. While short term Cd balance studies can give a measure of the amounts of Cd ingested and the major pathways of Cd excretion the technique is less suitable, because of the large daily variations in Cd excretion, for an accurate measurement of daily accumulation of Cd. A long term slaughter technique comparing the Cd content of various tissues of sheep slaughtered at 100 or 200 day intervals, which will give a more accurate value of the daily retention of Cd, showed that the daily accumulations of Cd were 1 and 3  $\mu$ g when the daily Cd intakes were 200 and 800  $\mu$ g respectively (Lee, Grace and Rounce unpublished data).

The net secretion of Cd into the stomach region most likely reflects the entry of Cd into the reticulorumen via the saliva. Faecal Cd is made up of unabsorbed dietary Cd and that from endogenous sources such as digestive tract secretions and spent mucosal cells. A preliminary estimation from

the Cd-106 enrichment of faeces and tissues indicated that the daily faecal endogenous Cd loss is about 1 µg/day (Lee unpublished data).

It has been well documented that the liver and kidney have the greatest propensity to store Cd, mainly as a Cd metallothionein complex (Bremner and Beattie, 1990). However as muscle and fat make up about 87% of the carcass, (Ulyatt and Barton, 1963) and as the amount of Cd associated with muscle (Table 2) and fat is small, the carcasses in contrast to the liver and kidney from sheep grazing high Cd pastures (e.g. 0.5 to 1 µg/kg DM) or low Cd pastures for 3 or 4 years will only accumulate small amounts of Cd and are therefore acceptable for human consumption. Other edible tissues also have lower, but variable Cd concentrations when compared to the liver and kidney. The provisional tolerable weekly intake of Cd for adults is 400 to 500 µg Cd (World Health Organisation, 1979).

Future studies on Cd in ruminants will (a) determine the faecal endogenous Cd loss and its true absorption using isotope dilution techniques with Cd-106 (b) measure the rate of uptake of Cd by the liver and kidney over a 2 year period and (c) define the relationship between the pasture (soil) Cd content and the accumulation of Cd in grazing livestock

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#### REFERENCES

- Bremner, I.; Beattie, J.H. 1990. Metallothionein and the trace minerals. *Annual Reviews of Nutrition* 10: 63-83.
- Faichney, G.J. 1975. The use of markers to partition digestion within the gastro-intestinal tract of ruminants. In: *Digestion and Metabolism in the Ruminant*. Eds. J.W. McDonald and A.C.I. Warner. The University of New England Publishing Unit, Sydney pp 277-292.
- Friberg, L.; Kjellstrom, T.; Nordberg, G.F. 1986. *Handbook on the Toxicity of Metals*. Vol. II Specific Metals. 2nd ed. Elsevier Science Publishers, Amsterdam p130.
- Lee, J.; Grace, N.D.; MacKay, A.D.; Hedley, M.J.; Rounce, J.R. 1993. Cadmium in soil, pasture and grazing ruminants. *Proceedings of the XVII International Grassland Congress*, Massey University (in press).
- Joblin, K.N.; Lee, J. 1990. Movement of nutrient and non-nutrient elements in the liquid phase in sheep rumen. *Journal of Animal Science* 68: 2067-2074.
- Mills, C.F.; Dalgarno, A.C. 1972. Copper and zinc status of ewes and lambs receiving increased dietary concentrations of cadmium. *Nature* 239: 171-173.
- Rothery, E. 1986. Analytical methods for Zeeman graphite tube atomizers. Varian Techatron Pty. Limited. Publication No. 83-100 650-80. Victoria, Australia.
- Sturgeon, R.E.; Berman, S.S.; Willie, S.N.; Resautniers, J.A.H. 1981. Pre-concentration of trace elements from seawater with silica-immobilized 8-hydroxyquinoline. *Analytical Chemistry* 53: 2337-2340.
- Syers, J.K.; MacKay, A.D.; Brown, M.W.; Currie, L.D. 1986. Chemical and physical characteristics of phosphate rock materials of varying reactivity. *Journal of the Science of Food and Agriculture* 37: 1057-1064.
- Tan, T.N., Weston, R.H., Hogan, J.P. 1971. Use of <sup>103</sup>Ru-labelled tris (1,10-phenanthroline) ruthenium (II) chloride as a marker in digestion studies with sheep. *International journal of applied radiation and isotopes* 22: 301-308.
- Ulyatt, M.J.; Barton, R.A. 1963. A comparison of the chemical and dissectible carcass composition of New Zealand Romney Marsh ewes. *Journal of Agricultural Science, Cambridge* 60: 285-289.
- World Health Organisation 1979. Environmental health criteria for cadmium: summary. W.H.O. Geneva.