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CADMIUM ACCUMULATION BY FORAGE SPECIES USED IN NEW ZEALAND LIVESTOCK GRAZING SYSTEMS

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Abstract

Little data exists on cadmium (Cd) accumulation in many plant species now commonly used as animal forages in New Zealand livestock grazing systems. A glasshouse trial was undertaken on 12 forage species to address this knowledge gap. Mean tissue Cd concentration decreased in the order chicory > plantain > turnip > lucerne > sheep's burnet > strawberry clover > kale > perennial ryegrass > haresfoot trefoil > red clover > crimson clover > white clover. Chicory and plantain had significantly greater mean tissue Cd concentrations (1.639 and 0.734 mg kg^{-1} DM, respectively) than all other species. Cadmium in ryegrass and white clover (0.103 and 0.035 mg kg^{-1}, respectively) was similar to that reported in other field and laboratory studies.

A survey undertaken across a range of commercial farms with varying soil type, land use and phosphorus (P)-fertiliser history validated the results of our glasshouse trial; chicory had a mean Cd concentration of 1.82 mg kg^{-1} (range 0.40-4.50 mg kg^{-1}) and plantain had a mean Cd concentration of 0.80 mg kg^{-1} (range 0.23-2.40 mg kg^{-1}).

Modelling of lamb kidney Cd accumulation indicated that food standard maximum levels may be exceeded in animals younger than the current meat industry 30 month offal discard age. With increased use of chicory and plantain as specialist forage crops in New Zealand, this information will be important for improving livestock Cd accumulation risk assessment models.

Introduction

Cadmium accumulation in New Zealand agricultural soils has been strongly correlated to history of P-fertiliser usage (Bramley, 1990; Roberts *et al.*, 1994). As Cd retains high bio-/phyto-availability in the soil for extended periods, and since plants have little ability to regulate Cd uptake, plant tissue Cd concentration tends to increase with increasing soil Cd concentration (Adriano, 1986; Smolders and Mertens, 2012). As elevated Cd in the human diet has been associated with human health disorders (Chaney *et al.*, 1999), Cd accumulation in agricultural land and plant species is undesirable.

In New Zealand agriculture, considerable research was carried out on plant Cd accumulation in the 1990s. Aside from a few significant food crops (potatoes, onions, lettuce and wheat (Roberts *et al.*, 1995; Gray *et al.*, 2001)) the majority of this work focused on ryegrass and legume based pastures (Roberts *et al.*, 1994; Loganathan *et al.*, 1995; Roberts and Longhurst, 2002; Gray and McLaren, 2005) given the importance of these species to our livestock grazing systems. These studies demonstrated Cd concentrations in ryegrass-white clover pasture systems to be relatively low (typically <0.3 mg/kg DM). Roberts *et al.* (1994) did however note that (non-specific) pasture weed species tended to contain greater Cd concentrations than both grasses and legumes. As livestock kidney / liver Cd accumulation is related to dietary Cd intake (Lee *et al.*, 1994; Lee *et al.*, 1996) a practical recommendation to manage Cd accumulation in grazing livestock is to minimize weed content in pastures, and to avoid grazing young stock (which accumulate Cd more rapidly) on Cd rich forages (Lee *et al.*, 1994; Lee *et al.*, 1996). To minimize risk of trade exposure from the potential exceedance of food standard maximum levels (MLs) for Cd, livestock offal is screened from shipments for human consumption for animals greater than 30 months of age (MAF, 2008).

Over the last two decades, considerable diversification in the forage species used in New Zealand livestock grazing systems has occurred, with a wide range of specialist forages now being used to fill feed deficits or provide higher quality feed (e.g. for finishing stock). Examples include the widespread use of winter and summer forage brassica crops (e.g. kale, swede and turnip) and herbs such as chicory and plantain. As there is no documented information on Cd accumulation in these forage species, we do not understand whether any of these forage plants pose risk to increased rates of livestock liver/kidney Cd accumulation, and whether this could increase exceedance of food standard MLs in offal products. Furthermore, this data is needed to improve existing animal Cd accumulation models (Reiser *et al.*, 2014). This research was undertaken as part of a wider study to improve our knowledge database on Cd accumulation in forage species used in modern livestock grazing systems, and to help quantify any potential risks associated. Data from the glasshouse trial and animal Cd accumulation modelling components of this paper represents a summary of the research presented by Stafford *et al.* (2016).

Glasshouse trial

A glasshouse trial was conducted to assess the relative Cd accumulation in 12 different forage plant species (Table 1). Soil was collected from the A-horizon of a commercial dairy farm near Balclutha, South Otago (characteristics described in Table 2) before being dried, sieved (<2 mm) and transferred into pots.

Table 1. Plant species assessed in the trial (reproduced from Stafford *et al.*, 2016)

Species		Cultivar	Approximate seed sowing rate (kg ha^{-1})
1	Perennial ryegrass (*Lolium perenne*)	Trojan	22
2	White clover (*Trifolium repens*)	Huia	8
3	Red clover (*Trifolium pratense*)	Hamua	15
4	Lucerne (*Medicago sativa*)	Wairau	15
5	Crimson clover (*Trifolium incarnatum*)	Blaza	15
6	Strawberry clover (*Trifolium fragiferum*)	Palestine	10
7	Haresfoot trefoil (*Trifolium arvense*)	Increase	6
8	Sheep's burnet (*Sanguisorba minor*)	-	10
9	Chicory (*Cichorium intybus*)	Puna II	5
10	Plantain (*Plantago lanceolata*)	Tonic	4
11	Kale (*Brassica oleracea*)	Gruner	4
12	Turnip (*Brassica rapa*)	Barkant	2

Potassium chloride and calcium sulphate (each at 500 mg/kg soil) were incorporated into the soil to supply basal potassium and sulphur, along with lime at 1400 mg/kg soil (equivalent to 2000 kg/ha) to correct soil pH to the agronomic optimum range of 5.8-6.0. Superphosphate (9.5% Total P, 9.0% P soluble in 2% citric acid, and 168 mg Cd/kg P) was ground and mixed into the potted soil at various rates to modify plant P and Cd availability, and plant growth rate. In addition to a control (zero superphosphate), plant species 1-10 received superphosphate rate treatments of 200, 400, 600, 800 and 1000 mg/kg soil (equivalent to 19, 38, 57, 76 and 95 mg P/kg soil, and 0.0032, 0.0064, 0.0096, 0.0128 and 0.0160 mg Cd/kg soil, respectively), while plant species 11 & 12 were limited to two superphosphate rates; 600 and 1400 mg/kg soil (equivalent to 57 and 133 mg P/kg soil, and 0.0096 and 0.0233 mg Cd/kg soil, respectively). Each forage species by superphosphate rate treatment had 4 replicates, except for the two brassica species (species 11 & 12; Table 1), which only had 3 replicates per treatment.

Table 2. Description and characteristics of soil used in the trial (reproduced from Stafford *et al.*, 2016)

Soil type			Waitahuna deep silt loam, rolling phase		
New Zealand Soil Classification (NZSC)			Mottled Fragic Pallic soil		
USDA Taxonomy			Typic Hapludalfs		
Organic matter (%)	Organic Carbon (%)	Total P (mg/kg)	Total Cd (mg/kg)	pH	Olsen-P (mg/L)
6.3	3.6	775	0.43	5.6	10

Trace elements (molybdenum, boron, cobalt and zinc) were applied periodically in the irrigation water, while nitrogen (N)-fertiliser was applied to pots containing the ryegrass and herb species (species 1 & 8-10; Table 1) at 50 mg/kg soil (approximately 25 kg N/ha) to alleviate N-deficiency that occurred in these species towards the end of the trial. All pots were then laid out in a glasshouse (mean temperature 15 °C, range 12-27 °C) in a randomized design. The two brassica species were harvested once only, 270 days after planting. Multiple harvests occurred for all other species, spaced approximately 5 weeks apart. The vegetative tissue of kale and turnip was separated into leaf and storage organ components for separate analysis. No separation of vegetative tissue occurred for other plant species.

Results

Mean tissue Cd concentration for each plant species is shown in Figure 1. The apparent order of Cd concentration (highest to lowest) in the tested plant species was chicory > plantain > turnip > lucerne > sheep's burnet > strawberry clover > kale > perennial ryegrass > haresfoot trefoil > red clover > crimson clover > white clover.

With the exception of lucerne and strawberry clover, the legume species and perennial ryegrass had mean tissue Cd concentrations ranging between just 0.035 and 0.103 mg/kg DM. These values are comparable to those reported previously for grass and legume based pastures in New Zealand glasshouse and field studies (Roberts *et al.*, 1994; Loganathan *et al.*, 1995; Lee *et al.*, 1996; Loganathan *et al.*, 1997; Gray *et al.*, 1999b, a; Roberts and Longhurst, 2002; Reiser *et al.*, 2014). Strawberry clover, lucerne, sheep's burnet, kale leaf and stem and turnip leaf and bulb all had moderate tissue Cd concentrations, ranging between 0.152 and 0.526 mg/kg DM. In comparison, chicory and plantain had much greater mean tissue Cd concentrations than all other species, at 1.639 and 0.734 mg/kg DM, respectively.

A common theme reported within the literature is that tissue Cd concentration is inversely related to plant yield (Mortvedt *et al.*, 1981; Loganathan *et al.*, 1997; Roberts and Longhurst, 2002). In our trial, for individual plant species plant tissue Cd concentrations were poorly correlated to plant yield (R^2 = <0.001-0.504) with a significant negative correlation evident only for kale leaf (Table 3). Although not significant, a number of plant species actually showed a trend for increasing tissue Cd concentration with increasing yield, possibly reflecting the slightly greater soil Cd phytoavailability with Cd inputs in the superphosphate (data not shown). However, for individual plant species, there was little correlation between soil total Cd concentration and plant tissue Cd concentration (R^2 = 0.006-0.428) with the relationship only significant for perennial ryegrass and red clover (Table 3). These poor correlations are likely influenced by the relatively narrow range of soil Cd values established by the various rates of superphosphate that were applied, meaning that inherent methodological and analytical error is more likely to mask detection of actual treatment differences.

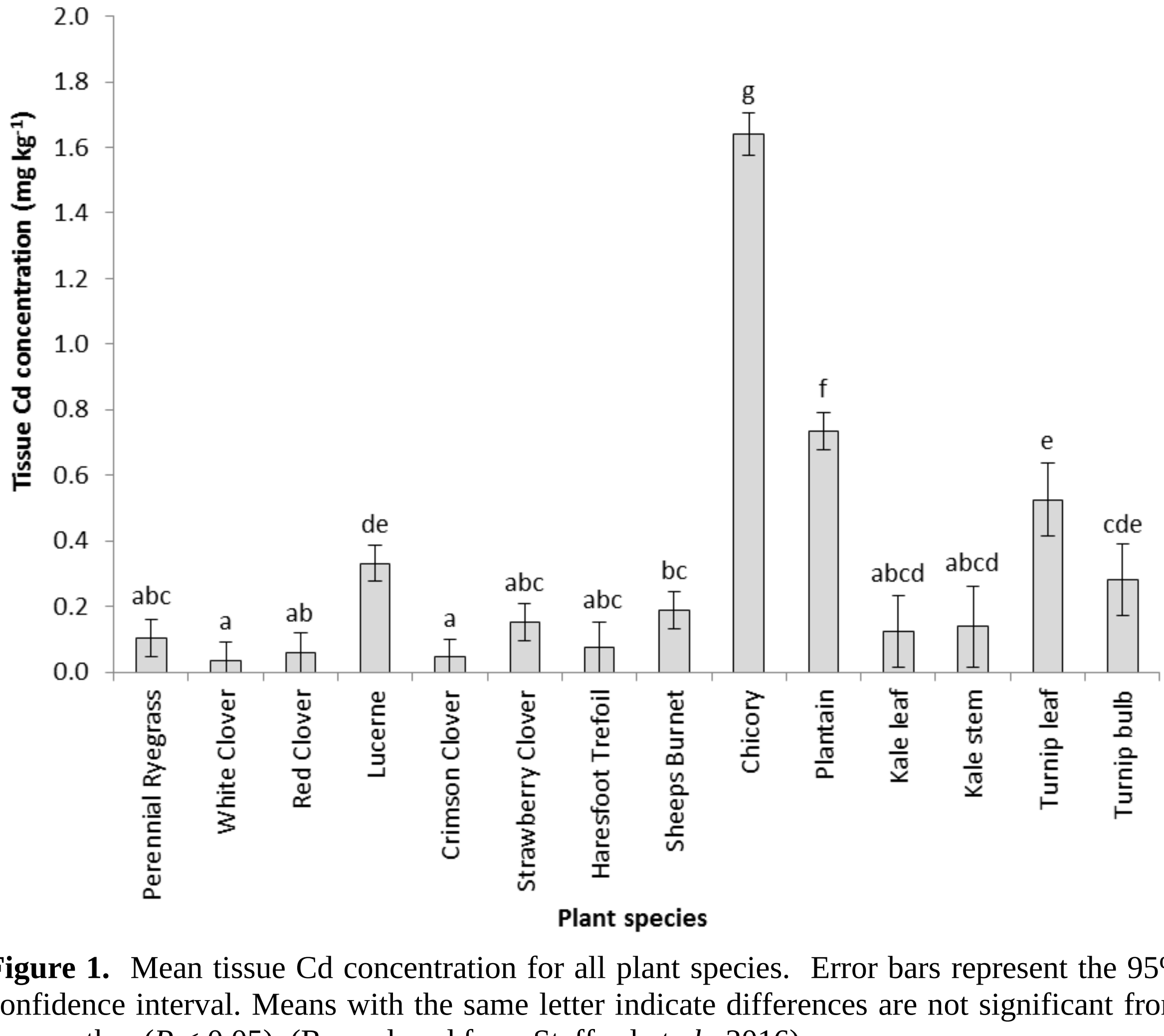

Figure 1. Mean tissue Cd concentration for all plant species. Error bars represent the 95% confidence interval. Means with the same letter indicate differences are not significant from one another ($P < 0.05$). (Reproduced from Stafford *et al.*, 2016)

Given Cd accumulation in the liver and kidney of livestock is strongly related to daily dietary Cd intake (Lee *et al.*, 1996) the high tissue Cd concentration of chicory and plantain is an important finding, since both species are increasingly used as specialist summer forage and livestock finishing crops (Somasiri *et al.*, 2015), commonly sown either in monoculture stands or in combination with legumes such as white and red clover. Although several previous studies have indicated the potential for these species to be Cd accumulators, the previous studies have lacked context, missing either species-specific data (Roberts *et al.*, 1994; Parker *et al.*, 2008), soil Cd data (Crush and Evans, 1990; Parker *et al.*, 2008) or data for other plant species that provides a comparative benchmark for the absolute Cd concentration presented (Crush and Evans, 1990).

Table 3. Linear regression coefficients of determination and significance for the relationship between plant Cd concentration (mg/kg DM) and **a)** plant yield (kg DM/ha), or **b)** soil total Cd concentration (mg/kg). (Reproduced from Stafford *et al.*, 2016)

Species	**a)** Plant yield		**b)** Soil total Cd	
	R^2	*P value*	R^2	*P value*
Perennial ryegrass (*Lolium perenne*)	0.009	*0.669*	0.222	*0.027*
White clover (*Trifolium repens*)	0.056	*0.289*	0.082	*0.195*
Red clover (*Trifolium pratense*)	0.028	*0.467*	0.215	*0.039*
Lucerne (*Medicago sativa*)	0.173	*0.054*	0.045	*0.332*
Crimson clover (*Trifolium incarnatum*)	0.003	*0.820*	0.053	*0.291*
Strawberry clover (*Trifolium fragiferum*)	<0.001	*0.945*	0.006	*0.741*
Haresfoot trefoil (*Trifolium arvense*)	0.276	*0.079*	0.004	*0.838*
Sheep’s burnet (*Sanguisorba minor*)	0.084	*0.203*	0.007	*0.709*
Chicory (*Cichorium intybus*)	0.026	*0.534*	0.055	*0.382*
Plantain (*Plantago lanceolata*)	0.119	*0.148*	0.013	*0.618*
Kale leaf (*Brassica oleracea*)	0.506	*0.032*	0.031	*0.651*
Kale stem *(Brassica oleracea)*	0.002	*0.910*	0.009	*0.825*
Turnip leaf (*Brassica rapa*)	0.189	*0.242*	0.124	*0.352*
Turnip bulb (*Brassica rapa*)	0.364	*0.085*	0.428	*0.056*

Field survey

To validate the findings of the glasshouse study, a survey of chicory and plantain crops was undertaken across thirty commercial dairy and sheep and beef properties. As the soil in our glasshouse study was of relatively low total Cd concentration, this survey also allowed us to understand Cd accumulation in these forage crops across a wider range of soil total Cd concentrations (driven by variation in historic land use and P fertilizer application), and across a wider range of soil types encompassing differences in soil pH, carbon content (% C) and clay mineralogy. Samples were taken from sites covering Allophanic, Organic, Gley, Pumice, Brown, Pallic and Recent soil orders.

In addition to chicory and plantain, plant tissue samples were also collected from ryegrass or white clover plants. Where possible, these were taken from within the same paddock. If this was not possible (e.g. a monoculture stand) these were taken from an adjacent paddock of the same soil type and landform with similar fertilizer history. These ryegrass and white clover samples were used to provide a benchmark for the Cd concentrations measured in the chicory and plantain samples. Each plant tissue sample represented a composite sample made up of 4 different plant tissue harvest points within each paddock (restricted to areas within paddock of common soil type / topography) with each harvest point typically comprising 3 chicory or plantain plants (or a similar mass of ryegrass or white clover). At each harvest point, 3 soil cores were collected from the area immediately adjacent to the harvested plants, with all soil subsamples from a site bulked together for analysis.

Results

Box and whisker plots for the plant tissue Cd concentration data for the different forage species are shown in Figure 2. Overall, trends in tissue Cd concentration for the different species are consistent with our glasshouse trial, with mean tissue Cd concentrations of 1.82, 0.80, 0.11 and 0.08 mg/kg DM for chicory, plantain, ryegrass and white clover, respectively. The Cd concentrations ranges for ryegrass and white clover in this field survey are similar to those reported by several other authors (Roberts *et al.*, 1994; Loganathan *et al.*, 1995; Lee *et al.*, 1996; Loganathan *et al.*, 1997; Gray *et al.*, 1999b, a; Roberts and Longhurst, 2002; Reiser *et al.*, 2014). Clearly evident is the large range in plant tissue Cd concentrations for chicory and plantain. In contrast, variation within the plant tissue samples for ryegrass and white clover is relatively narrow.

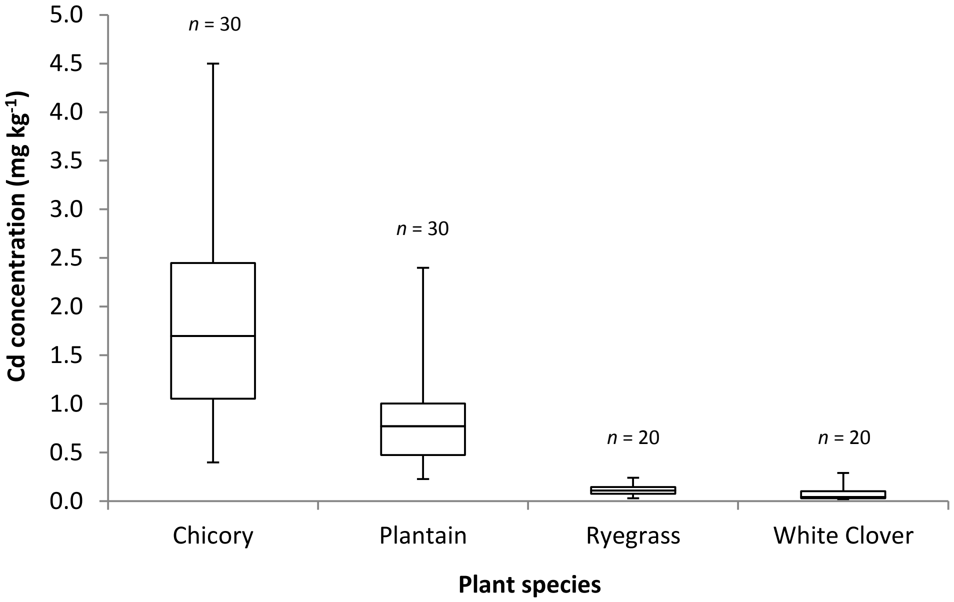

Figure 2. Tissue Cd distribution by species across the properties sampled. Box and whiskers represent data quartiles.

The large variation in tissue Cd concentration for chicory and plantain indicates the sensitivity of these plant species to factors which influence soil Cd phytoavailability. For both chicory and plantain, regression analysis indicated that there was a significant relationship plant tissue Cd concentration and the combined soil variables total Cd concentration, pH, and % carbon (Table 4). However, the coefficient of determination was poor for plantain (R^2 = 0.305) in contrast to chicory (R^2 = 0.745) indicating other factors were responsible for a larger proportion of the variability in plantain tissue Cd concentrations. In contrast to chicory and plantain, there was no relationship between ryegrass or white clover tissue Cd concentration and the combined variables soil total Cd, pH and % carbon (Table 4).

Table 4. Linear regression coefficients of determination and significance for the relationship between plant tissue Cd concentration (mg/kg DM) and the soil factors total Cd, pH and % carbon (combined, and individually).

Plant species	R^2	*P-value*			
		Overall	tCd	pH	%C
Chicory	0.745	*<0.001*	*<0.001*	*0.001*	*0.001*
Plantain	0.305	*0.022*	*0.042*	*0.078*	*0.460*
Ryegrass	0.308	*0.108*	*0.022*	*0.817*	*0.235*
White clover	0.086	*0.687*	*0.930*	*0.938*	*0.303*

For chicory, there was a significant positive correlation between soil total Cd concentration and plant tissue Cd concentration (Table 4) with this factor alone explaining 55% of the variability in tissue Cd concentration (data not shown). As would be expected based on known principals of soil Cd sorption (McBride *et al.*, 1997; Gray *et al.*, 1999c; Loganathan *et al.*, 2012) soil pH and % carbon were significantly negatively correlated to chicory Cd concentration (Table 4) and so further tightened the coefficient of determination when combined, however this relationship was not apparent for the other species in this dataset.

Animal modelling

An existing lamb kidney Cd accumulation model (Lee *et al.*, 1996) was used to investigate the potential impact of the plant Cd concentrations from our glasshouse trial on animal Cd accumulation. The model is summarised below:

$$[Cd]_{kidney} = (-205 + 0.981Cd_{intake} + 0.726Time) \div 1000$$

where $[Cd]_{kidney}$ refers to kidney Cd concentration (mg Cd/kg FW), Cd_{intake} represents livestock daily dietary Cd intake (μg Cd/day), and *Time* is the period (days) over which livestock are exposed to this daily dietary Cd intake.

We modelled three different forage scenarios for lamb finishing; 1) chicory monoculture, 2) chicory-white clover forage stand, 3) ryegrass-white clover pasture (Table 5). A weighted-average forage Cd concentration for the mixed forages was applied using the Cd concentrations from our glasshouse trial and assumed sward percentages for each species (Table 5). Further to this, other key assumptions in our modelling approach were a lamb daily feed intake of 1 and 1.5 kg DM/lamb/day, coupled with grazing periods on these forage intakes of 60 and 30 days, respectively.

Based on these inputs, the model of Lee *et al.* (1996) predicts lamb kidney Cd concentration to exceed the current European Commission (EC) food standard ML of 1.0 mg/kg FW (EC, 2014) for both chicory monoculture scenarios, and also for the chicory-white clover mixed stand on the higher daily feed intake scenario. In comparison, the model predicts little change to lamb kidney Cd concentration on the ryegrass-white clover forage. The current New Zealand ML of 2.5 mg/kg FW (FSANZ, 2011) is not breached in any scenario.

Table 5. Variables used in determining kidney Cd concentration for lambs finished on different forages, using the equation of Lee *et al.* (1996). (Reproduced from Stafford *et al.*, 2016)

	Chicory monoculture forage stand		Chicory - white clover forage stand		Perennial ryegrass - white clover pasture	
Cd concentration in chicory (mg Cd/kg DM)	1.639		1.639		-	
Cd concentration in ryegrass (mg Cd/kg DM)	-		-		0.103	
Cd concentration in white clover (mg Cd/kg DM)	-		0.035		0.035	
Proportion of chicory in sward (%)	100%		60%		-	
Proportion of perennial ryegrass in sward (%)	-		-		75%	
Proportion of white clover in sward (%)	-		40%		25%	
Cd concentration in diet (mg Cd/kg DM)	1.639		0.997		0.086	
Daily feed intake (kg DM/lamb/day)	1.0	1.5	1.0	1.5	1.0	1.5
Cd_{intake} (µg/day)	1639	2459	997	1496	86	129
Time (days)	60	30	60	30	60	30
$[Cd]_{kidney}$ (mg Cd/kg FW)	*1.45*	*2.23*	*0.82*	*1.28*	*-0.08*	*-0.06*

These scenarios highlight the sensitivity of the (Lee *et al.*, 1996) model to daily intake of Cd, which is a function of feed intake (kg DM/day) and Cd concentration in the feed (mg/kg DM). Because of this sensitivity, the model would suggest that elevated Cd concentrations can occurred in short periods of time on high Cd feed, which could result in more frequent exceedance of food standard MLs. It should be recognised however, that the Cd concentrations in chicory and plantain in this modelling scenario fall well outside the calibration dataset upon which the original model was constructed, and therefore the model may not be reliable. Furthermore, as chicory and plantain are known to accumulate high concentrations of other divalent metals such as zinc (Harrington *et al.*, 2006) that there may be competitive interaction between such elements that may limit the sorption of Cd in the liver / kidney (Parker *et al.*, 2008). If this were the case, then the question arises; what happens to the Cd if it is not sorbed into kidney / liver tissues - is more simply excreted in the dung, or is it expressed in other ways by the animal? Further animal grazing studies are recommended to understand animal Cd accumulation and cycling on Cd-rich chicory and plantain forage crops.

Summary

The data presented within this research shows that plant species commonly used as specialist forages within modern livestock grazing systems vary widely in their ability to accumulate Cd in their vegetative tissues. In particular, chicory and plantain are able to accumulate significantly greater Cd concentrations relative to traditional ryegrass-white clover pastures. As Cd accumulation in livestock liver and kidney has previously been shown to be related to daily dietary Cd intake, risk assessment modelling indicates that grazing livestock on these Cd-rich forages has potential to increase the exceedence of food standard MLs for Cd in animal offal products. However, animal grazing trials are required to validate the predictions of the existing animal Cd accumulation models, since the plant tissue Cd concentration data for chicory and plantain lies well outside the original calibration datasets that were used to develop these models. Future work should also look to evaluate the effect of modifying soil pH as a practical means of manipulating Cd accumulation in these Cd-rich forages.

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