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EFFECT OF CADMIUM APPLICATION AS A HEAVY METAL ON DRY MATTER INTAKE AND ITS DIGESTIBILITY AND ABSORPTION OF CADMIUM IN SHEEP *

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ABSTRACT

An experiment was conducted to examine the effect of cadmium on digestibility and intake of feeds and the gastrointestinal absorption of cadmium (Cd) application in sheep.

Twenty four (24) Welsh Mountain ewes were used in the experiment. The ewes were blocked into six groups by weight and allocated at random. The average animal weight for each block was 50.1, 47.9, 46.7, 45.0, 40.4 and 38.9 kg, respectively. Each treatment had 12 replicates. Grass contaminated with cadmium was produced. Treatment 1 consisted of grass with cadmium plus 200 g concentrates and Treatment 2, the Cd-free grass and 200 g concentrates containing 5 mg of Cd. Animals were kept in metabolism crates for thirty days, the first 15 days being an adaptation period. During the adaptation period grass was fed *ad libitum* while approximately 200 g of concentrates were offered daily.

There were significant ($P < 0.05$) effects of the treatments on the total dry matter feed intake. However, cadmium effect on dry matter digestibility almost reached statistical significance ($P = 0.06$). Cadmium came out in the faeces mostly on the second and third day, which has confirmed the previous reports. The absorption rate of cadmium was not calculated due to interferences with sodium in the cadmium determinations. The available equipment (Atomic Absorption Spectrophotometer, AAS-model 151) had no background correction for all non-specific absorption.

Key Words : Heavy metals, cadmium, dry matter intake, digestibility, absorption.

ÖZET

AĞIR METAL OLARAK KADMİUMUN KOYUNLARDA KURU MADDE TÜKETİMİNE VE SİNDİRİLEBİLİRLİĞİNE ETKİLERİ VE KADMİYUM ABSORPSİYONU

Bu deneme kadmiumun, koyunlarda yemlerin sindirilebilirliğine, kuru madde tüketimine ve absorbsiyonuna olan etkilerini incelemek amacıyla yürütülmüştür.

Denemede yirmi dört (24) adet Welsh Mountain ırkı koyunlar kullanılmıştır. Hayvanlar ağırlıklarına göre tesadüfi olarak altı (6) gruba ayrılmış ve muamaleler tesadüfi

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olarak gruplara tahsis edilmiştir. Her bir grupta ortalama hayvan ağırlığı sırasıyla 50.1, 47.9, 46.7, 45.0, 40.4 ve 38.9 kg'dır. Her bir muamele 12 tekerrürden oluşmuş olup 1. muamele kadmiyum kontamineli çayır otu ve 200 g konsantre yem içermekte; 2. muamele is kadmiyumsuz çayır otu ve 5 mg kadmiyum içeren 200 g konsantre yemden oluşmaktadır. Hayvanlar 15 günü adaptasyon periyodu olmak üzere 30 gün metabolizma sandıklarında tutulmuştur. Adaptasyon periyodu süresince 200 g konsantre yem verilirken çayır otu *ad libitum* olarak verilmiştir.

Muameleler toplam kuru madde tüketimini önemli derecede (P< 0.05) etkilemiştir. Ancak, muamelelerin kuru maddenin sindirilebilirliğine olan etkileri istatistiki olarak önemlilik derecesine yakın bulunmuştur (P=0.06). Yapılan analizler sonucunda, kadmiyumun yemlemenin ikinci ve üçüncü gününde dışkıda belirlenmiş olması diğer çalışmaları teyid etmektedir. Kadmiyumun absorbsiyon oranı, kadmiyum tayininde sodyumla karışık olması yüzünden hesaplanamamıştır. Denemede kullanılan Atomik Absropsiyon Spektorofotometre' si (model 151) spesifik olmayan absorbsiyonlar için yeterli okumalara sahip değildi.

Anahtar Kelimeler : Ağır metaller, kadmiyum, kurumadde tüketimi, sindirilebilirlik, absorbsiyon.

INTRODUCTION

The accumulation of cadmium in agriculture systems of some countries such as in New Zealand (Bramley, 1990) as a result of extensive use of phosphatic fertilisers has been recognised as a potential problem following the appearance of unacceptable levels of cadmium in some animal products.

Cadmium (Cd) is a non-essential trace element and is considered as a pollutant. Cadmium pollution in the environment occurs from smelting industries, attrition of automobile tyres, and burning of diesel and heating oil. Cadmium is added to soils with phosphatic fertilisers and with sewage sludge application to agricultural land. Soil and the above-ground parts of plants also receive additions through the atmosphere, particularly in areas near metal smelters (Bozkurt and Zachou, 1999).

Several factors affect the activity of cadmium in soils and its availability for plant uptake such as soil pH; the amount of Cd present; the metal sorption capacity of the soil; the presence of micro-elements and of macro-nutrients; soil temperature, moisture content and soil aeration (Bozkurt and Zachou, 1999).

Certain skin penetration of soluble cadmium compounds can take place when they are applied as a solution to the skin. Skin is not though the main route of cadmium uptake. The respiratory and gastrointestinal tracts are the two main routes of absorption in man and animals.

Airborne cadmium is a potential danger for animals through its deposition to grassland which is grazed by them and not through direct inhalation. Cadmium is translocated through the food chain of soil, roots, vegetation and animals to man and is an accumulative poison and in daily life large populations of animals and man are exposed to low levels of cadmium in the environment and in food. Cadmium accumulation in pasture becomes increasingly a problem because of its potential transfer into higher food chains. Increased Cd levels in grass means potential cadmium accumulation in animals and health hazards for the consumer.Therefore, an experiment was set up to investigate the absorption rate of cadmium in sheep and cadmium effect on dry matter intake and dry matter digestibility of feeds. The study included production of grass following foliar application of hydrous cadmium nitrate ($Cd(NO_3)_2.4H_2O$). The experiment was carried out at the University farm of Universiy of Wales, Bangor, UK between June and November 1994.

METHODS AND MATERIALS

Herbage production

The experiment was conducted in 22 concrete lysimeters of a green house. The dimensions of these were 1.25m length and 1.1m width (1.375 m^2). They were full of sand due to previous experiments. The top 10 cm of sand was cleared and 5-7.5 cm of top soil was added. The added soil had been used for other trials. The last experiment involved a cereal crop which is a very demanding crop in terms of nutrients. Thus, the soil used (mixture of sand and top soil) in this experiment was of low fertility.

Phosphorus and potassium fertiliser (0:24:24, P and K compound fertiliser) was applied at the rate of 40 kg/ha. The pots were sown with 30 kg/ha of perennial ryegrass (*Lolium perenne spp.*) in mid-June 1994.

Due to sunny dry days that followed sowing the plots were covered with top soil and netting to reduce drying of soil. Germination started a week later. The germination ratc was low due to the dry conditions and disturbance by birds. Empty patches of soil were resown with no big improvement of the germination rate due to unfavourable weather conditions. The grass was weeded regularly to avoid competition for nutrients.

Grass growth was encouraged with two nitrogen dressings applied at the rate of 40 kg/ha early and late July 1994.

Grass sampling

Grass sampling was carried out as described by Zachou and Bozkurt (1999). The grass was cut manually on three successive days (19, 20, 21/9/94). Grass sub-samples from each lysimeter were weighed and oven dried at 80°C for 36 hours. The grass of all lysimeters for each treatment was mixed and subsequently sampled in order to determine the cadmium level of the grass fed to the sheep. The samples were weighed again after drying for their dry matter content. Grinding in a hammer mill followed so that the samples could pass through a one millimetre screen. The grass was then stored in sealed polythene bags for determination of Cd, Ca, Mg, and K (MAFF, 1986). Chemical analysis for Cd, Ca, Mg and K content of grass was performed as described by MAFF (1986).

Animal management

Twenty four (24) Welsh Mountain ewes were used in the experiment. The animals had been purchased one and a half months before the beginning of the trial. They were grazing in one of the university farm's field during this period, without any concentrate supplementation. The ewes were kept in good health throughout the experiment.

Experimental design

A dairy shed, 18 m long and 9.5 m wide, was used to accommodate the ewes. The shed had four doors. Three of them were kept closed at all times. No artificial lighting or ventilation was used. One of the longest side walls of the building was partially covered with 5 mm hard board. The building was divided into six blocks, each containing four metabolism crates. The blocks were arranged in relation to possible environmental differences within the shed with regard to a door at one end and one side with partial enclosure.

The ewes were blocked into six groups by weight and the groups were allocated at random to the six blocks within the building. The average animal weight for each block was 50.1, 47.9, 46.7, 45.0, 40.4 and 38.9 kg, respectively.

The metabolism cages were placed in the two corridors of the shed; eight (8) on one side and sixteen (16) on the other side. The crates were put in the passageway because the existing cubicles were on a slope and they would make impossible the separation of faeces from urine.

Nine (9) metabolism cages were of an old design described by Vera (1985). The rest were modified in order to accommodate bigger breeds than Welsh mountain sheep in the future and to work more efficiently.

Data collection and Treatments

A part (2.5 ha) of the field of the university farm was kept ungrazed for the experiment. The field was seeded, 5-6 years ago, with a mixture of grass species, predominantly ryegrass. However, the grass sward contained some clover as well. The field was fertilised with 70 kg N/ha in February 1993 and with 60 kg N/ha and 30 kg K/ha in end of August 1993. The grass was cut every 2-3 days with a small Agria cutting machine (model 5400) depending on the weather conditions during the experiment. The grass produced for daily consumption was kept in the shed and the rest was kept in a cool room for the next 1-2 days.

Faeces were separated from urine by a curved metal plate and collected in an aluminium tray while urine was collected in a plastic pot. Splash boards were fitted in the back of the cages to avoid mainly urine spillage. Faeces were collected for 15 days after the first day of treatment while urine collection stopped after the sixth day.

Every morning grass residues were collected before feeding and were oven dried. The grass that was fed was sub-sampled for determination of its dry matter content. The daily intake was calculated as follows:

- the fresh weight of the grass was converted to dry weight by multiplying with its dry matter content
- the weight of the concentrates was converted to dry weight by multiplying with its dry matter content
- the dry weight of the grass was added to the dry weight of the concentrates. The dry weight of the residue was subtracted from the above mentioned value.

All calculations were based on dry weight.

Each treatment had 12 replicates. The ewes were put in the cages 15 days before the beginning of the feeding for adaptation. One ewe died during the adaptation period. During that adjustment period grass was fed *ad libitum* while approximately 200 g of concentrates were offered daily. Only during the adaptation period, concentrates were offered to the animals every morning before the grass was fed so that they would get used to eating them. The grass produced was enough to feed the animals for one and a half days. The composition of the concentrates is shown in Table 1.

Table 1. Composition of the concentrates

Ingredients	% of DM
Sunflower	25
Orange citrus	20
Maize gluten	15
Sugar beet	10
Palm kernal	10
Grass nuts	10
Molasses	7.5
Minerals plus vitamins	2.5

The concentrate supplement contained 10000 IU/kg Vitamin A, 2000 IU/kg Vitamin D3, 20 IU/kg Vitamin E and 0.35 mg/kg sodium, selenite, selenium. The stated levels of the vitamin activity would remain effective for three months if the concentrates were stored in a cool and dry place. The DM content of the concentrate supplement was 86% and also contained 17% protein, 3% oil, 13% fibre, 5% starch and 9% sugar.

TREATMENT 1

Treatment 1 consisted of grass with cadmium plus 200 g concentrates. The first day the ewes were fed 2 kg of grass (fresh weight) and 200 g of concentrates. On that day the feeding was restricted because the intake was usually higher than 2 kg of grass. This was done to ensure the same cadmium intake for all ewes. The second day, ewes were fed with 1.25 kg of grass containing Cd, 200 g concentrates and then grass *ad libitum* for the rest of the day. Feeding was taking place in regular base to ensure that animals had grass in front of them at all times.

Ewes were fed the Cd-grass first and then the concentrates to ensure that they ate all the offered grass, spesifically for the animals in this treatment. Following the two days, the animals were fed grass *ad libitum* from a field of the University Farm.

Treatment 2

In Treatment 2 sheep were fed with the Cd-free grass described above and 200 g concentrates containing 5 mg of Cd. To avoid dust the cadmium, as hydrous cadmium nitrate ($Cd(NO_3)_2.4H_2O$), was dissolved in water. One millilitre of the cadmium solution was added to the concentrates with a pipette. Each millilitre contained 5 mg of cadmium. The concentrates were fed in plastic pots to avoid any spillage. 200 g of concentrates and 2 kg of Cd-free grass were fed during the first day. The second day the ewes were fed 200 g of concentrates with 5 mg of Cd, 1.25 kg of Cd-free grass and then *ad libitum* grass cut from the field. The following days the animals were fed with 200 g concentrates and grass *ad libitum*.

Faeces and urine sampling and chemical analysis

1-Faeces and urine sampling

Faeces and urine were collected daily. Urine was collected from 10 to 11 am., while faeces from 11 am to 1 pm., starting always from the same cage to ensure 24 h interval between two subsequent samples. Urine sub-samples were stored in glass bottles for Cd determination. The urine of the first six days was tested for its cadmium content. No Cd was detected in the urine, so the urine sampling stopped after the sixth day of the experiment. Faeces were collected for fifteen (15) days after cadmium was administered.

The total faeces collection was recorded and subsequently sub-sampled. The sub-samples were oven-dried at 80°C for three days. The fresh and dry weight of faeces' sub-samples was recorded in order to calculate their dry matter content.

The sub-samples of the faeces were ground in a hammer mill so that they could pass through a one millimetre screen. The samples were stored in sealed polythene bags for determination of their cadmium content.

2-Cadmium content of faeces samples

The method described as in (MAFF, 1986) was used to extract the Nitric-Perchloric acid soluble cadmium in faeces samples. Two (2) grammes of faeces were weighed and finally diluted to 20 ml volumetric flask. Anti-static spray was used during weighing because of static electricity of the samples.

Each sample was duplicated and a blank determination was carried out as well. The faeces extract was kept in plastic bottles and stored in a cool room until determination of their cadmium content with an Atomic Absorption Spectrophotometer.

Statistical procedure

The results from this experiment were statistically analysed using the statistical package Minitab (Windows, version 10). A Shapiro-Wilk test was also performed to examine if the data represented a population with a normal distribution. T-test was applied to test if the cadmium concentration of the grass was significantly different from zero (0).

Analysis of variance was performed using GLM (General Linear Model) to examine differences in mineral content of grass, in grass yield, in dry matter intake and in digestibility between treatments. Days were used both as fixed factor and covariate.

The statistical model was as follows:

$$Y_{ij}=\mu+\alpha_i+\varepsilon_{ij}$$

Y_{ij}=dependent variable, mineral content of grass or grass yield, or dry matter intake or digestibility

μ= is the overall population mean

α_i= is the main effect of the i' *th* level of cadmium

ε_{ij}=is the random effect independent

RESULTS

Cadmium availability

All results were tested for their normality and they were all normally distributed. Soil was sampled after cutting the grass for its mineral and cadmium content in previous experiment (Zachou and Bozkurt, 1999). The aim was to assess the availability of cadmium to the plants and also the effect of calcium, magnesium and potassium on cadmium availability.

Cadmium content of grass

The grass was digested by wet ashing to avoid cadmium which can be high in dry ashing (Gordon *et al.*, 1971). Wet digestion of grass samples (two from each lysimeter) took place after the feeding trial in sheep.

The cadmium content of the grass was measured as described by (MAFF, 1986). The cadmium level of grass was tested twice (five and twenty four days after the last cadmium application) employing dry ashing and a recovery rate test. Cd-free grass samples were weighed acurately. Known concentration of cadium solution was added to these samples in order to test the recovery rate after dry ashing. The cadmium concentration of grass was 11 mg/kg with a recovery rate varying from 45 to 63%.

The dry matter intake was calculated so that each sheep in the first treatment involving feeding Cd complexed in grass would get 5 mg of cadmium in the diet. The appropriate calculations were made to ensure 5 mg of Cd in the concentrates so that the experiment would be balanced.

After analysing the grass with the wet digestion method it was discovered that the grass contained on average only 5.93 mg/kg of Cd resulting in an unbalanced experiment.

The Cd concentration of grass was significantly different ($P < 0.01$) from zero. This was tested by performing a t-test. No cadmium was detected for the control treatment. This was expected as there was no Cd in the soil (Zachou and Bozkurt, 1999) and Cd application was done very carefully to avoid Cd contamination of the control lysimeters.

Cadmium effect on grass yield

Cadmium had no significant ($P > 0.05$) effect on grass yield as explained in detail in a previous experiment (Zachou and Bozkurt, 1999). The yield (both fresh and dry matter yield) and the DM% of the grass was unaffected by the treatment as it is shown in Table 2.

The fresh yield varied from 3.04 to 4.7 kg and from 2.70 to 5.1 kg for the control and the Cd-treatment, respectively. The mean fresh weight of the Cd-free grass was 3.8 kg. The mean fresh weight of the Cd-treated grass was 3.5 kg.

Table 2. Effect of cadmium application on yield and dry matter content of grass*.

		Cadmium				
	n	With	s.e	Without	s.e	P
Fresh yield (kg)	11	3.5	0.064	3.8	0.064	0.30
DM yield (kg)	11	0.6	0.006	0.6	0.006	0.61
DM (%)	11	17.8	0.172	16.4	0.172	0.09

*The values presented in the table are not arithmetic means. They are obtained by GLM analysis.

The dry matter content of the grass varied from 15.4 to 18.9% and from 14.9 to 20.5% for the Cd-free and the Cd-grass, respectively. The mean DM content was 16.4 and 17.8% for the control and the Cd treatment respectively.

The dry matter of the yield for the Cd-free grass varied from 0.47 to 0.72 kg giving a mean value of 0.62 kg. The dry weight of the yield for the Cd-treated grass varied from 0.52 to 0.76 kg with a mean value of 0.61 kg. These results are in accordance with previous reports (Sadana *et al.*, 1989; Strnad *et al.*, 1991) using low levels of cadmium.

Total dry matter feed intake

The mean daily dry matter intake during the experimental period for Treatment 1 and 2 is shown in Figure 1.

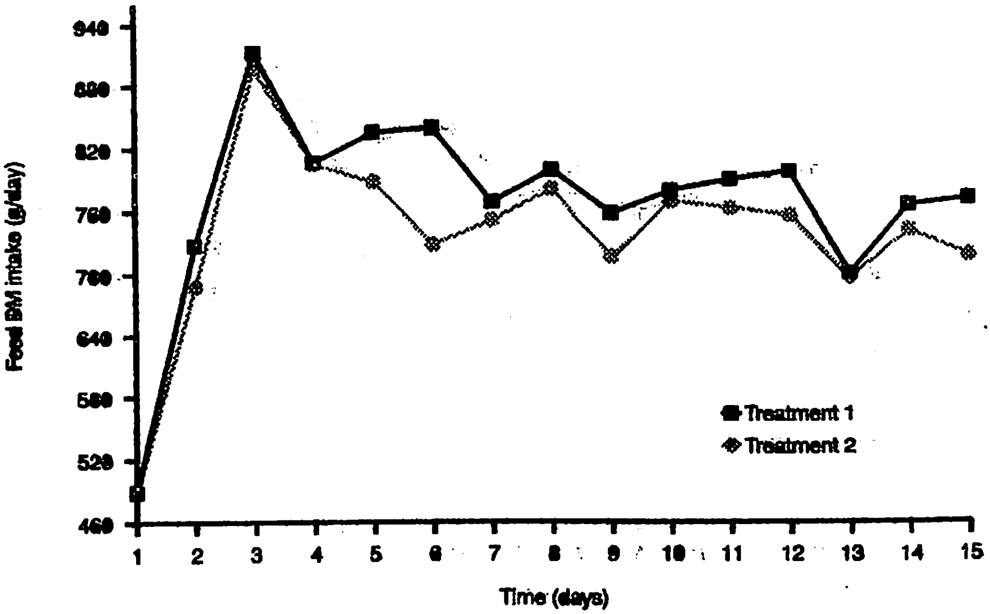

Figure 1. Mean daily dry matter intake for Treatment 1 and 2

The dry matter intake was significantly different between the treatments (Figure 1). The first day, the mean dry matter intake was 488.2 g and 489.3 g for the Treatment 1 and 2 respectively. The intake was restricted as described earlier (in the *treatments* section) during the Day 1. The intake varied from 699.9 to 913.2 g for Treatment 1 and from 688.1 to 897.7 for Treatment 2 during the experiment after the first day.

The results of analysis of variance performed to examine the effect of treatments on the dry matter intake (DMI) and dry matter digestibility (DMD) are shown in Table 3. Days were used both as fixed factor and covariate in this analysis.

Table 3. Effect of treatments on dry matter intake and digestibility

Treatments	n	DMI (g)	s.e	P	DMD (g)	s.e	P
1	165	764.4	0.71	0.044	73.11	0.03	0.06
2	180	738.9	0.65	0.044	74.19	0.03	0.06

Treatment 1 significantly ($P < 0.05$) increased the dry matter intake. The treatment effect on dry matter digestibility almost reached statistical significance ($P=0.06$). It seems that Treatment 2 with cadmium administered in the concentrates tended to have higher digestibility than Treatment 1 where cadmium was complexed in the grass.

The dry matter intake of the animals in both treatments tended to be significant ($P=0.052$) between days while there was no difference ($P=0.527$) in the digestibility of the diet between days.

Absorption rate of cadmium

Faeces extracts had high sodium content resulting in higher total concentration than expected. The absorption rate of cadmium could not be calculated because of this.

Part of the sodium interference effect was eliminated by analysing faeces sampled previously to give blank readings.

The results showed that most of the Cd came out in the faeces on the second and third day after receiving the Cd dose (Figure 2). Cadmium analysis was not carried out after the ninth day due to low readings. This is in accordance with the results of Miller *et al.* (1968) where the highest excretion rate was observed the second day after a single oral dose.

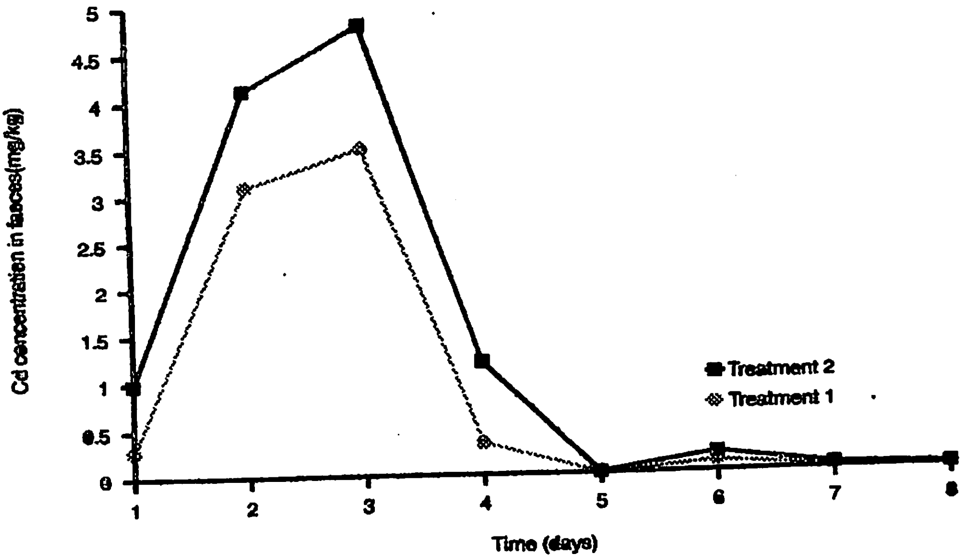

Figure 2. Daily faecal excretion of cadmium by sheep in Treatment 1 and 2.

DISCUSSION

Cadmium application did not significantly affect grass yield which was discussed in detail by Zachou and Bozkurt (1999). This finding is in accordance with the previous experiments when low concentrations of cadmium were used. Strnad *et al.* (1991) reported no yield reduction of clover (*Medicago sativa*) when 0.2 mg Cd/kg of soil were applied in combination with 10 mg Pb and 10 mg Cu/kg of soil.

It is generally observed that low cadmium application (mg of Cd/kg of soil) can affect crop yields while DM yield decreases dramatically at higher rates of cadmium application (Zachou and Bozkurt, 1999).

A randomised block design was employed not only to investigate the cadmium effect on dry matter digestibility and intake but also to test the absorption rate of cadmium in sheep. Animals were blocked into six groups by weight. The design leads to confounding of weight and environmental effect, but this was accepted because the effect of weight was not to be examined in this study. The aim was to detect any differences in the absorption rate of Cd in sheep when cadmium is complexed in herbage in comparison with directly applied Cd to the feed. For this reason any age differences were not accounted in the statistical analysis as well.

The experiment resulted in to be unbalanced between the two treatments but this has been reported in the past as well. Several investigators (Chaney *et al.*, 1978;

Furr *et al.*, 1976; Hanson and Hinesly, 1979) have studied cadmium uptake from sewage sludge by plants; edible parts of these plants were then fed to experimental animals. In all cases the higher dietary cadmium was not matched in the experimental design by controls with a higher inorganic cadmium source.

Significant differences were found in the total dry matter feed intake between Treatment 1 and Treatment 2. Treatment 1, where cadmium was complexed in the grass had significantly higher dry matter intake when days where used both as fixed factor and covariate. The dry matter intake tended to differ significantly between days.

The dry matter digestibility of the diet tended (P=0.06) to be higher in Treatment 2 while there was no significant difference on the dry matter digestibility between days. Sheep in both treatments were fed the same amount of concentrates (200 g) and grass *ad libitum* (except the first day) so no differences were expected due to the treatment.

Unfortunately, for reasons that have been mentioned previously it was not possible to calculate the absorption rate of cadmium in sheep. It is generally agreed that the gastrointestinal retention of cadmium is low. Most of the Cd is excreted in the faeces. This was demonstrated in this study as well, where no Cd was detected in the urine.

No blood tests were carried out to assess the Cd-status of the ewes after treatment. It was judged unnecessary based on previous reports. Earlier attempts (Friberg *et al.*, 1974) to determine levels of radioactive cadmium in blood after a single dose have not been successful.

Doyle *et al.*, (1974) reported no appreciable increase in blood cadmium when cadmium (5, 15, 30 ppm) was fed in growing lambs for 191 days. They believed that sheep have the ability to regulate blood Cd when relatively high levels of cadmium were fed.

Similarly, Smith *et al.* (1991) found that there was no treatment effect on packed cell volume, haemoglobin, total serum protein, numbers of red and white blood cells, plasma glucose, blood urea nitrogen, serum creatinine and glutathione peroxidase when cows were fed with 0.25, 1 or 5 ppm of Cd for 394 days.

Cadmium seems to affect the metabolism of essential elements causing deleterious effects on animals. Cadmium absorption from a single oral dose is small. The liver and the kidney appear to be the two organs of the greatest interest with regard to cadmium storage. However, Cd is found in most compartments of the body.

Animals are intermediate receptors in the food chain. Extensive research on cadmium accumulation and absorption is needed to assess cadmium as a health hazard due to difficulties concerning research with human subjects.

Widespread concern has been increasing to maintain agricultural sustainability. Therefore, it is inevitable that the toxicity of cadmium and other heavy metals in terms of human health should bring the issue to the attention of producers, scientists and policy-makers as well.

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