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Transfer of cadmium, lead, mercury and arsenic from feed into tissues of fattening bulls: chemical and pathological data

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Abstract

Transfer effects of the ingestion of known amounts of cadmium, lead, mercury and arsenic were examined with young bulls, fattened under Dutch circumstances. The ration was maize silage and concentrates (2:1 on dry matter base). The animals were slaughtered at 500 kg body weight. The elements were administered to the ration as soluble compounds or via harbour or sewage sludge. Also experiments with cadmium-rich feed phosphate and maize silage have been performed.

Increasing dietary concentrations of cadmium, lead, mercury and arsenic resulted in significantly higher concentrations of these elements in the liver, and particularly, in the kidney. Cadmium, administered as acetate (about 12 mg kg^{-1} dry matter) even doubled the cadmium concentration in muscle tissue. In the other experiments, transfer of cadmium, lead and mercury from feed into muscle tissue was negligible. The arsenic concentration in muscle tissue remained low, although a higher dietary concentration resulted in an increase.

The highest dose of soluble lead, mercury and arsenic to the ration was equal to the maximum permitted concentration as set by EEC-Directives (10 mg lead, 0.1 mg mercury and 2 mg arsenic per kg). The highest supplemented dose of cadmium equaled that of lead. These maximal dietary concentrations resulted in 6.9 mg Cd, 1.7 mg Pb, 0.08 mg Hg and 0.17 mg As per kg fresh tissue of kidney and 2.6 mg Cd, 0.86 mg Pb, 0.024 mg Hg and 0.10 mg As per kg fresh tissue of liver. Gross and histopathological lesions have been observed, but these were not related to the concentration of the elements in the ration, because these lesions have also been found for control bulls (without supplementation of elements to the ration).

Introduction

In a previous study (Vreman et al., 1986) results were reported about the carry-over of cadmium, lead, mercury and arsenic from feed into milk and tissues of lactating dairy cows. Similar experiments with fattening lambs have been described (van der Veen et al., 1986). Although various carry-over experiments with beef cattle have been published (Bertrand et al., 1981; Boyer et al., 1981; Hofmann et al., 1981; Johnson et al., 1981; Baxter et al., 1982; Kreuzer et al., 1982; Ray et al., 1982; Rundle et al., 1984), it was considered necessary to obtain data with young bulls, fattened under Dutch circumstances. In the Netherlands, fattened bulls are commonly slaughtered at a body weight of about 500 kg. This meat-producing livestock is mainly fed on maize silage and concentrates (2:1 on dry matter base). The study described was designed to obtain information on the levels of the mentioned elements in beef and edible organs which correspond with legal limits in the whole ration. The different forms, which were available and have been administered to the concentrate of the daily ration of fattening bulls, were: (a) soluble compounds, (b) harbour or sewage sludge, (c) cadmium-rich feed phosphate and (d) cadmium-rich maize silage. This silage was made from maize, grown on clay soil with high cadmium concentrations. Other practical sources for animal feed, containing high toxic element concentrations, could not be found in sufficient quantities.

Materials and methods

Animals and administration of elements

For practical reasons (housing capacity of the experimental farm; other feeding experiments at the same time; supply of heavy-metal-rich raw materials), four separate trials (one per year) have been conducted.

Trial 1 comprised two groups: 8 bulls were used as control group, and 16 bulls received additional quantities of cadmium, lead, mercury and arsenic in a soluble form (experimental group) for the last five months of the fattening period until slaughter. Arsenic was added as As_2O_5 and the other elements were added as their acetates. The compounds were dissolved in water and pipetted on cattle cake. The added quantity was such that the concentration in the whole ration (maize silage plus concentrates), based on dry matter, was about 10 mg cadmium, 10 mg lead, 0.1 mg mercury and 2 mg arsenic per kg. These concentrations for lead, mercury and arsenic corresponded with the maximum permitted concentrations in the regulations of the Commodity Board for Feeding Stuffs (1975). For cadmium, a tolerance level had not yet been established. Further details are summarized in Table 1. The bulls were also used in an other feeding experiment to study the effect of high and low feed and energy intake on growth and feed conversion. It was therefore possible in this trial to distinguish between a low and a high feed intake group, which were both low in heavy metals in their control ration.

Trial 2 involved 18 bulls, which were allocated at random into 3 groups of 6 animals each. The two experimental groups were offered a ration with either dry sew-

Table 1. Summary of experimental data.

	Number of bulls	Experimental period (days)	Mean growth (g day^{-1})	Mean daily feed intake (kg DM)	
				concentrates	maize silage
Trial 1					
Control	8	143			
L[1]			1045	1.3-1.8	3.4-4.8
H			1220	2.1-3.3	3.9-5.8
Experimental[2]	16	143			
L			1043	1.3-1.8	3.4-4.8
H			1205	2.1-3.3	3.9-5.8
Trial 2					
Control	6	177	1130	2.1-3.3	3.9-5.8
Harbour sludge	6	174	1128	2.3-3.6	3.9-5.8
Sewage sludge	6	174	1123	2.3-3.6	3.9-5.8
Trial 3					
Control	8	328	1174	1.7-3.4	1.6-4.6
Phosphate[3]	8	328	1125	1.7-3.4	1.6-4.6
Trial 4					
Control	6	330	1182	2.0-3.3	1.4-4.7
Phosphate	6	330	1176	2.0-3.3	1.5-4.7
Maize silage[4]	6	330	1131	2.0-3.3	1.8-5.2
Phosphate + maize silage	6	330	1187	2.0-3.3	1.6-4.7

[1] L = low energy intake group; H = high energy intake group.
[2] Soluble compounds.
[3] Feed phosphate with high cadmium.
[4] Maize silage with high cadmium.

age sludge or dry harbour sludge. The sludges were incorporated into the concentrates (70 kg per ton). The sludge feeding period comprised the last 6 months of the growing stage before slaughter. The third group was a control group. Further details are summarized in Table 1.

Trial 3 was conducted with 16 young bulls, which were divided into two groups of eight animals each. The control group received concentrates, containing a feed phosphate with low cadmium (<1 mg kg^{-1}) and the experimental group was fed on concentrates with high cadmium concentration in the used feed phosphate (on average 45 mg kg^{-1}). This treatment was continued for 11 months until slaughter. Other details are given in Table 1. This experiment was performed to gather only data on the transfer of cadmium.

Trial 4 included 24 fattening bulls, which were allocated at random into 4 groups of 6 animals each. The control group was fed on maize silage and concentrates, both

Table 2. Composition of concentrate pellets used in the various trials and of premix (in %).

	Trials 1, 3, 4	Trial 2
Concentrate pellets		
Dried beet pulp	42	39
Soy bean meal	32	30
Wheat middlings	10	9
Cane molasses	10	9
Minerals (premix)	5.5	5.5
Sludge	–	7.0
Premix		
Limestone	4.63	
Calcium phosphate	12.0	
Calcium magnesium phosphate	42.0	
Iodized salt (NaCl)	40.0	
Manganese sulphate	0.11	
Zinc sulphate	0.75	
Copper sulphate	0.12	
Cobalt sulphate	0.015	
Sodium selenite	0.001	
Vit. A and D_3	0.375	

with low cadmium concentration. The first experimental group received concentrates with more cadmium due to the incorporation of cadmium-rich feed phosphate. The second experimental group was fed on concentrates with low cadmium and on maize silage with relatively high cadmium (1.5-2.0 mg kg^{-1} dry matter), originating from cadmium-rich soil (10-15 mg kg^{-1}). The third experimental group was fed on concentrates and maize silage, both with high cadmium. Other details are given in Table 1. Trial 4 referred, like Trial 3, only to cadmium.

The animals of all trials, which were mainly of MRY-breed, had been housed in the stanchion barn for fattening bulls of the Institute for Livestock Feeding and Nutrition Research at Lelystad. The total ration, based on dry matter, consisted for about one-third of concentrates and two-thirds of maize silage, which was fed ad libitum. Tap water was freely available. The composition of concentrates and the premix are given in Table 2. Concentrations of elements in concentrates and maize silage are listed in Table 3. The calculated concentrations of elements in the total daily ration were based on the data of Table 3 and on the proportion of concentrates and maize silage in the total daily ration. The concentrations of elements in drinking water were negligible (Cd: ≤ 0.001; Pb: <0.01; Hg: 0.0001 and As: ≤ 0.01 mg kg^{-1}).

Observations and sampling

The bulls were weighed at monthly intervals and at the start and end of the experi-

Table 3. Concentrations of selected elements (mg kg^{-1} DM) in concentrates and maize silage; mean ± standard deviation.

	Cd	Pb	Hg	As
Trial 1				
Concentrates (n = 4)	0.3 ± 0.2	1.4 ± 0.3	<0.02	<0.2
Maize silage (n = 2)	0.1 ± 0	4.7 ± 0.5	0.01 ± 0	0.5 ± 0.1
Trial 2				
Concentrates with harbour sludge (n = 3)	1.0 ± 0.1	15.5 ± 2.1	0.2 ± 0.1	2.6 ± 0.7
Concentrates with sewage sludge	0.8 ± 0.1	21.3 ± 3.1	0.12 ± 0.03	1.1 ± 0.3
Concentrates without sludge (n = 1)	0.4	1.6	<0.01	0.2
Maize silage (n = 6)	0.10 ± 0.02	2.2 ± 0.2	<0.01	0.30 ± 0.04
Trial 3				
Concentrates with Cd-rich phosphate (n = 3)	1.3 ± 0.1			
Concentrates with Cd-low phosphate (n = 2)	0.4 ± 0			
Maize silage (n = 3)	0.1 ± 0			
Trial 4				
Concentrates (Cd-low phosphate, n = 7)	0.2 ± 0.1			
Concentrates (Cd-high phosphate; n = 7)	1.2 ± 0.1			
Maize silage (Cd-low; n = 6)	0.1 ± 0.1			
Maize silage (Cd-rich; n = 6)	1.8 ± 0.3			

ments. Throughout the experimental periods, various samples were taken from concentrates, maize silage and tap water to determine the intake of elements. In the fourth trial, blood and urine were also sampled to determine chemical and particularly biochemical parameters (see section 'biochemical analyses'). At the end of the experimental period the bulls were slaughtered and samples of various tissues and organs were taken, particularly muscles, liver and kidney.

Chemical analyses

The preparation of samples and chemical methods were as previously described (Vreman et al., 1986) and performed at the State Institute for Quality Control of Agricultural Products (RIKILT) at Wageningen. Comparison of means for liver and kidney between groups was accomplished by analysis of variance after log transformation of original values.

Biochemical analyses

Biochemical analyses were only done in blood and urine of bulls from Trial 4. Blood was analysed for haemoglobin, packed cell volume, red cell counts, leucocytes, alcaline phosphatase, lactatedehydrogenase, sorbitol dehydrogenase, γ-glutamyltranspeptidase, urea, creatinin, total protein, albumin and α-, β- and γ-globulin. Urine was analysed for protein and glucose. These analyses were performed at the Laboratory of the Clinic for Inner Diseases of the Faculty of Veterinary Medicine, using routine procedures.

Methods of pathological examination

These methods were similar to those previously described (Vreman et al., 1986); only samples of 2 untreated (no additional elements) and 4 treated bulls of Trial 1 and of 4 untreated and 4 treated bulls (1.6 mg dietary cadmium per kg dry matter) of Trial 4 were examined. After slaughter, gross inspection was done of liver, kidney, spleen, heart, skeletal muscles and testicles. Specimens of these organs were collected for histological examination using standard techniques.

Results and discussion

Throughout the trials the fattening bulls were in good health and the growth rates (on average 1.1-1.2 kg day^{-1}) were normal for young bulls fed on maize silage and beet pulp-soy bean concentrates.

The results of the chemical analyses are presented in Tables 4-7.

Effect of dietary Cd on Cd concentrations of different tissues

Increasing dietary Cd resulted in most cases in significantly higher concentrations of Cd in liver and, particularly, in kidney. Exposure to relatively high dietary Cd (11.6 mg kg^{-1} DM) doubled on average the Cd concentration in muscle. No significant increases were observed in muscle Cd concentration of bulls of the other experimental groups. The Cd concentrations in similar tissues of bulls from the low and the high feed intake group in Trial 1 were not significantly different. Compared with the results of the bulls fed on concentrates with Cd-rich feed phosphate the increase in organ Cd concentration of sludge-fed bulls of Trial 2 was less pronounced. Cd concentrations in brain, thymus, heart, testis and bone were highest for the experimental group of Trial 1 and averaged 0.010, 0.026, 0.046, 0.045 and 0.050 mg kg^{-1} fresh weight, respectively.

According to an EEC directive (Anon., 1987), the maximum permitted concentration of Cd in complete feeding stuffs for ruminants, with the exception of complete feeding stuffs for calves, lambs and kids, has been set at 1 mg kg^{-1} of feed with 12 % moisture. It follows from the data of Table 4 that this level in the whole diet will lead to mean Cd concentrations in kidney, liver and muscle of about 2.0, 0.5 and 0.002 mg kg^{-1} fresh weight, respectively. These concentrations are below the

Table 4. The effect of different dietary Cd concentrations (mg kg^{-1} DM) on Cd concentrations of tissue samples (mg kg^{-1} fresh tissue).

Groups	Ration[1]	Kidney			Liver			Muscle		
		n[2]	mean	s.e.m.[3]	n	mean	s.e.m.	n	mean	s.e.m.
Trial 1										
Control	0.2	4	0.24	0.05	4	0.08	0.01	18[4]	0.007	0.001
Exp.	11.6	8	6.9***	0.9	8	2.6***	0.3	39[4]	0.016***	0.001
Trial 2										
Control	0.2	6	0.19	0.03	6	0.04	0.01	2	0.003	0.001
HS[5]	0.4	6	0.33**	0.04	6	0.09*	0.02	2	0.002	0.001
SS	0.4	5	0.26	0.03	6	0.07*	0.01	2	0.002	0
Trial 3										
Control	0.2	8	0.26	0.02	8	0.09	0.01	2	0.004	0.001
Exp.	0.6	8	0.99***	0.08	8	0.43***	0.03	2	0.005	0.001
Trial 4										
Control	0.2	6	0.32	0.04	6	0.07	0.01	5	0.001	0.000
Phosphate	0.6	6	0.98***	0.07	6	0.28***	0.02	6	0.002	0.000
Maize	1.2	6	2.1***	0.2	6	0.61***	0.06	5	0.002	0.000
Phosphate + maize	1.6	6	2.0***	0.3	6	0.67***	0.06	6	0.003	0.000

1 Ration = mean Cd concentration in mg per kg DM of the whole ration.
2 n = number of samples.
3 s.e.m. = standard error of mean.
4 Muscle samples from 3 sites on the carcass: upper arm, diaphragm and tail head.
5 HS = harbour sludge; SS = sewage sludge.
* Significantly different ($P < 0.05$) from control; ** $P < 0.01$; *** $P < 0.001$.

legal limits of 3.0, 1.0 and 0.05 mg kg^{-1} fresh weight of kidney, liver and muscle, respectively (Anon., 1985).

In the present study, the cadmium concentration of the control diets was within the range of cadmium concentrations found in concentrates for dairy cattle (Vos et al., 1986). The mean cadmium concentration in dairy cattle concentrates of the national survey was 0.13 mg kg^{-1} feed as such (about 88 % dry matter). The mean cadmium concentration in maize of another national monitoring study was 0.43 mg kg^{-1} DM and the single values varied strongly, which was mainly due to variation in soil cadmium concentrations (Wiersma et al., 1986).

The results for cadmium in liver and kidney of control bulls are in agreement with data reported by Krása et al. (1986) from a carry-over experiment with fattening bulls and by Vos et al. (1987) from a monitoring programme investigating the levels of heavy metals in meat, liver and kidney of beef cattle.

The present study also shows that the bio-availability of cadmium in cadmium-rich feed phosphate is equal to that in cadmium-rich maize silage. A twofold higher

Table 5. The effect of different dietary Pb concentrations (mg kg^{-1} DM) on the Pb concentrations of tissue samples (mg kg^{-1} fresh tissue).

Groups	Ration	Kidney			Liver			Muscle			Bone		
		n	mean	s.e.m.	*n*	mean	s.e.m.	*n*	mean	s.e.m.	*n*	mean	s.e.m.
Trial 1													
Control	3.6	4	0.64	0.05	4	0.50	0.07	18	0.07	0.01	4	1.8	0.2
Exp.	15.0	8	1.7***	0.1	8	0.86*	0.09	39	0.09	0.01	4	4.9***	0.5
Trial 2													
Control	1.9	6	0.42	0.06	6	0.12	0.01	2	0.02		3	1.1	0.2
HS[1]	7.5	6	0.74**	0.07	6	0.47***	0.06	2	0.02		3	1.9***	0.03
SS	9.9	5	0.68**	0.02	6	0.32***	0.02	2	0.02		3	2.1***	0.2

[1] HS = harbour sludge; SS = sewage sludge.
* Significantly different from control ($P < 0.05$); ** $P < 0.01$; *** $P < 0.001$.

cadmium concentration in the ration evoked a twofold higher cadmium concentration in liver and kidney. The difference between the mean liver cadmium concentrations of bulls from the cadmium-rich phosphate group of Trial 3 and Trial 4 can not be explained.

Effect of dietary Pb on Pb concentrations of different tissues

It can be seen from Table 5 that the Pb concentrations in kidney, liver and bone are positively related to the dietary Pb concentrations. In contrast, the Pb concentrations in muscle tissue were low in all groups and no increases were observed in the experimental groups receiving the Pb-supplemented rations. The mean Pb concentrations in brain, thymus and testis were mostly slightly higher than those in muscle and no effect of increased dietary lead concentration was found.

The maximum permitted concentration of lead in complete feeding stuffs has been set at 5 mg kg^{-1} of feed with 12 % moisture. This dietary level will produce Pb concentrations in beef kidney, beef liver and beef muscle which are lower than the legal limits of 1.5, 1.0 and 0.4 mg kg^{-1} fresh weight, respectively. The Pb concentrations of the unsupplemented ration for the control groups were similar to data found for dairy cattle concentrates (Vos et al., 1986). In another national monitoring study the mean Pb concentration in whole crop maize was 2.2 mg kg^{-1} DM (Wiersma et al., 1986).

Ruminants can tolerate high dietary Pb concentrations, up to 1000 mg kg^{-1}, for several months without visible signs of toxicosis (National Academy of Sciences, 1980), but the maximum tolerable dietary level for Pb is considered to be 30 mg kg^{-1} for most species of domestic animals.

Effect of dietary Hg on Hg concentrations of different tissues

The elevated dietary Hg concentrations caused only significant increases in liver

Table 6. The effect of different dietary Hg concentrations (mg kg^{-1} DM) on the Hg concentrations of tissue samples (mg kg^{-1} fresh tissue).

Groups	Ration	Kidney			Liver			Muscle		
		n	mean	s.e.m.	*n*	mean	s.e.m.	*n*	mean	s.e.m.
Trial 1										
Control	0.01	4	0.014	0.006	4	0.008	0.003	18	0.004	0.001
Exp.	0.11	8	0.08***	0.01	8	0.024**	0.003	39	0.005	0.001
Trial 2										
Control	<0.01	6	0.005	0.0003	6	0.002	0.0004	2	0.001	0
HS[1]	0.08	6	0.068***	0.008	6	0.026***	0.009	2	0.002	0.001
SS	0.05	5	0.020***	0.001	5	0.008***	0.002	2	0.001	0

[1] HS = harbour sludge; SS = sewage sludge.
* Significantly different from control ($P < 0.05$); ** $P < 0.01$; *** $P < 0.001$.

and, particularly, in kidney Hg concentrations (Table 6). The maximum permitted concentration for whole feeding stuffs has been legally set at 0.1 mg kg^{-1} of feed with 12 % moisture. The highest mean concentrations of Hg in kidney, liver and muscle found in the present study were 0.08, 0.026 and 0.005 mg kg^{-1} fresh weight, respectively. The legal limits for Hg in these foods of animal origin are 0.1, 0.05 and 0.05, respectively.

Johnson et al. (1981) studied also the Hg retention in tissues of beef cattle fed on a diet containing 11.5 %, on dry matter basis, of sewage sludge. The experimental diet contained 2.6 mg total Hg per kg dry weight, while the Hg concentration of the control diet was below the limit of detection (<0.02 mg kg^{-1}). After 106 days of sewage sludge feeding, the Hg concentrations in liver and kidney of experimental bulls were significantly increased and amounted on average 0.27 and 2.04 mg kg^{-1} DM respectively, or 0.08 and 0.41 mg kg^{-1} fresh weight. This calculation was based on an estimated dry matter content of liver and kidney of 30 % and 20 %, respectively. Tissues like brain and bone showed no detectable Hg, while Hg concentration in spleen, lung and muscle tissues were slightly increased.

Effect of dietary As on As concentrations of different tissues

Increasing dietary As concentrations resulted in significant increases of As concentrations in kidney, liver and muscle tissue. Arsenic concentrations in brain, thymus, heart, testis and bone were mostly slightly lower than those in muscle tissue. The maximum permitted arsenic concentration in whole feeding stuffs has been set at 2 mg kg^{-1} of feed with 12 % moisture. Legal limits for As in foods of animal origin have not been established. The mean As concentrations in kidney, liver and muscles, due to a total dietary As concentration of 2.7 mg kg^{-1}, were 0.17, 0.10 and 0.05 mg kg^{-} fresh weight, respectively. These concentrations exceed the levels, which were found in a Dutch monitoring study between 1980 and 1985 (Vos et al., 1987).

Table 7. The effect of different dietary As concentrations (mg kg^{-1} DM) on the As concentrations of tissue samples (mg kg^{-1} fresh tissue).

Groups	Ration	Kidney			Liver			Muscle		
		n	mean	s.e.m.	*n*	mean	s.e.m.	*n*	mean	s.e.m.
Trial 1										
Control	0.5	4	0.06	0.02	4	0.022	0.004	18	0.011	0.001
Exper.	2.7	8	0.17*	0.03	8	0.10***	0.01	39	0.046***	0.003
Trial 2										
Control	0.3	6	0.04	0.01	6	0.008	0.002	2	0.003	0
HS[1]	1.3	6	0.10***	0.01	6	0.034***	0.006	2	0.012	0
SS	0.6	5	0.07***	0.02	6	0.018***	0.004	2	0.006	0.002

[1] HS = harbour sludge; SS = sewage sludge.
* Significantly different from control ($P < 0.05$); ** $P < 0.01$; *** $P < 0.001$.

Biochemical and pathomorphological examination

The results of biochemical analyses showed no effects of dietary Cd on any of the parameters studied during the whole experimental period of Trial 4.

Gross lesions

On gross inspection, chronic inflammatory foci were found in the liver of treated as well as untreated bulls. The kidney showed focal to diffuse interstitial nephritis with calculi in the pyelum and medulla. In the testicles white foci were seen in the parenchyma, and the heart showed diffuse endocardial haemorrhages. The remaining screened organs and tissues (skeletal muscles, lungs, thymus and spleen) showed no gross lesions. The observed lesions were found in treated (with supplemented Cd, Pb, Hg and As in Trial 1 or with Cd only in Trial 4) as well as control bulls.

Histopathological lesions

The liver lesions consisted of tiny granulomas in the parenchyma, and portal mononuclear cell infiltrates with fibrosis and occasionally eosinophils. The lesions in the kidney consisted of periglomerular fibrosis, mononuclear cell infiltrates combined with microcalculi in tubuli collecti and medullar interstitium. These were calcium deposits and had often induced necrosis of tubular epithelium. The macroscopically observed calculi in the pyelum in part of the bulls had led to a mononuclear pyelitis. The white foci in the testicles consisted also of calcium deposits, in part of the bulls accompanied by slight diffuse fibrosis. Sarcosporidiosis was found in the heart and skeletal muscles. The other screened organs showed no lesions or only lesions due to the slaughter process.

The observed lesions were found in treated (with supplemented Cd, Pb, Hg and

As in Trial 1 or with Cd only in Trial 4) as well as control bulls. The observed gross and histological lesions can not be considered as related to the oral administration of the investigated metals, as they were equally found in treated as well as control bulls. The renal lesions like calcium deposits with tubular necrosis may be attributed to the feed regime with high energy intake. The inflammatory lesions found in liver and kidney can be considered as from infectious origin, in the liver most likely of parasitic, in the kidney presumably of bacterial or viral nature. Other changes like sarcosporidiosis and calcium deposits in the testicles may be routinely found in fattening bulls.

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