DIOXIN CONCENTRATION IN MILK, FAECES AND TISSUES OF COWS RELATED TO FEED CONTAMINATION

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Introduction

Dioxin contamination of feed and food is a frequently discussed problem, even in the press. The EU legislation sets maximum levels for dioxins in food and feeding stuffs. The dioxin concentration of milk, faeces and tissues of lactating cows grazing in an area of higher dioxin level in the soil and grass, has been tested in part 1 of this small scale field study.

Soil intake of grazing cows can reach 1.5 kg soil/d under extreme meteorological conditions (1). Considering that, the dioxin load of soil and the degree of contamination of feedstuffs could play an important role regarding the exposition of grazing animals. Due to repeated flooding (2) the area the experiment took place was known for a higher dioxin level in soil and grass. The area was separated in a depression and an elevation (in flooding areas depressions are known for their higher dioxin level). Grassland is not only used for grazing animals, but also to obtain hay or grass silage. In part 2 of the field study grass silage obtained on the same area, was fed to dry cows to test whether or not there a risk for milk quality after parturition.

The primary aim of this field study was to investigate different matrices at different moments of the experiment. The secondary aim was to develop recommendations on how to prevent or at least how to minimize the dioxin load of feed and with it of food, if food producing animals graze on such areas.

Materials and Methods

Part 1 of the field study was carried out with three lactating cows (3-4 years old). After a clinical examination the dioxin concentration of a milk sample of each cow was ascertained to make sure, that they were not loaded before the trial.

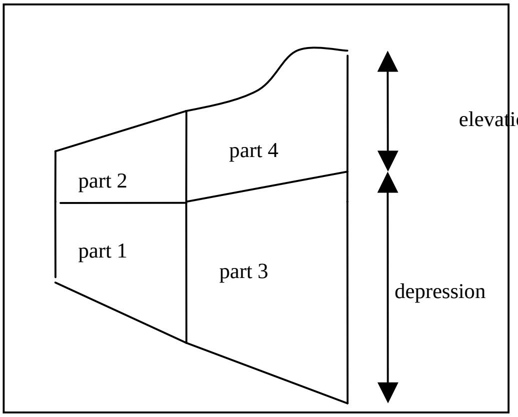

Fig.1: Investigation area

The three cows grazed for ten weeks on an area (Fig. 1), known for its higher dioxin level of the soil (2). Before the study started, the dioxin contents of soil of to of the parts and grass samples were examined. The area was separated in four parts and cows grazed in principle of rotational pasture (grass almost as exclusive feed; they were fed only about 100 g of concentrates to attract cows at milking time). During the grazing period, further grass samples were taken and

Pooled and individual milk samples were taken and frozen to be able to analyze their dioxin concentration later. In order to pursue the development of dioxin level in milk, pooled milk samples were examined after one, four and eight weeks. When the results were in hand, the kinetics could be estimated and individual milk samples were examined. At the end of the grazing period, rectal samples of faeces were taken and their dioxin concentration was also analyzed. Following the grazing period the cows were housed and fed with unloaded feedstuffs (total mixed ration and hay as basic feed) that dioxin level in milk samples would decrease. Management of milking and sampling was retained, and at the end of the final ten weeks when the cows were housed the dioxin concentration of further faeces samples were determined.
Three gravid cows were available for part 2 of the trial. One milk sample of each cow was taken before the dry period and examined to secure, that milk was not dioxin loaded before. After that the cows were dried off. Four weeks before their probable calving date, feedstuffs (up to this point unloaded total mixed ration and hay) were changed. Now cows were fed exclusively with dioxin contaminated grass silage obtained on the investigation area, parts 3 and 4 (also concentrates were fed, raised to calving date from 1 to 3 kg/cow/d). After parturition (cow 21:

31, cow 22: 34 and cow 23: 25 days after change of feedstuffs) cows were milked twice a day and they got unloaded feedstuffs again. Milk samples were taken and frozen to establish their dioxin level. Following milk samples were analyzed: first milk (colostrum), milk at the third day and milk three weeks post parturition. After 40/43/58 p.p. dioxin level of further milk samples were tested and concentrates were reduced for one week (7 kg before, now 1kg /cow/d) to provoke a mobilization of fat due to reduced energy supply. Herewith should be found out whether or not the dioxin concentration in milk would increase due to fat mobilization. After the end of trial cows were slaughtered and samples of muscle, liver and fat tissue were also tested on their dioxin level.

Analyses were done as follows:

The cream of the milk samples was separated; freeze dried and soxhlet-extracted with hexane. The fat extract was spiked with ^{13}C-labeled PCDDs/PCDFs. The hexane was evaporated and the remaining extract was additionally used for gravimetric fat determination. Muscle and liver samples were freeze dried and the fat was extracted with toluene. Feed samples were freeze dried (if necessary) and powdered, spiked before extraction and extracted with isopropanol/toluene (69/31, w/w). For the clean-up, the following steps were used: chromatography on a multi-layer column (2 g silica, 5 g silica/1 n NaOH (2/1 w/w), 2 g silica, 15 g silica/conc. sulphuric acid (11/9 w/w), 5 g silica/$AgNO_3$, 4 g Sodium sulfate), elution with 350 ml hexane. The residue after hexane evaporation was purified by chromatography on a carbon column (0,36 g Envicarb mixed with 1,64 g celite), forward elution with 50 ml cyclohexane/ethylacetate (1/1, v/v) for removing lipids, backward elution with 40 ml toluene yielding the PCDDs/PCDFs. This was followed by chromatography on 5 g Alumina B Super 1 for dioxin analyis (elution with 40 ml pentane, 75 ml hexane/dichloromethane 98/2 (v/v) and last with 50 ml hexane/dichloromethane 50/50 (v/v) containing the PCDD/PCDF.

Analysis was carried out with a HRGC/HRMS-system (Carlo Erba Mega 5000 with split/splitless injector directly coupled to a VG Autospec Ultima) at 10000 resolution. For separation of the PCDD/PCDF a DB-XLB (50 m x 0,25 mm x 0,25 µn) was used. GC temperature program: 403°K for 1 min, with 20 K/min to 493°K, with 1,5 K/min to 523°K, and with 20 K/min to 593°K, hold for 20 min. Injector temperature 503°K, transfer line temperature 523°K, carrier gas He at 1 ml/min, injection volume 1.5 µl in splitless mode with 60 s splitless period.

Results

Table 1 shows, that the dioxin concentration in soil on the investigation area (Fig. 1) was quite raised. A maximum level is not provided in German legislation. According to German Guidelines (3) agricultural use of the soil with dioxin levels above 40ng/kg DM should be avoided. The PCDD/F- concentrations in grass samples, which were taken during part 1 of the investigation were not as high as expected (referred to most samples), especially in consideration of the values of milk and faeces samples. Dioxin levels of grass silage used for part 2 of the trial, obtained in depression of the investigation area, were higher than dioxin levels of silage taken on elevation (Table 1).

There was no strong correlation between dioxin concentration and data of HCl-insoluble ash (degree of contamination of feedstuffs).

Table 1: Analyzed data in soil (Dioxin concentration), grass and grass silage (Dioxin concentration, dry matter and HCl- insoluble ash)

sample	part	time	laboratory	Dioxin concentration*	DM**	HCl- insoluble ash***
soil	1	beginning	LUFA	570		
soil	2	beginning	LUFA	464		
grass	1	beginning	LUFA	0.34	18.2	1.12
grass	2	beginning	LUFA	0.31	23.6	1.78
grass	2	middle	LUFA	0.56	34.8	1.31
grass	1	end	LUFA	0.42	33.2	4.00
grass	3	middle	LVL	2.5	35.8	3.65
grass	4	middle	LVL	1.1	43.1	4.03
grass	1/3/4	mixture	LUFA	0.82	32.2	3.89
grass	1/3/4	mixture	LVL	0.72		
grass silage	depression	beginning	LUFA	4.64	43,1	2.72
grass silage	elevation	beginning	LUFA	2.26	49.4	1.58
grass silage	elevation	middle	LVL	1.7	34.8	1.78

*soil: in ng WHO PCDD/F-TEQ /kg DM
grass: in ng WHO PCDD/F-TEQ /kg DM (rel. to 88% DM)
maximum level (feedstuffs legislation): 0.75ng TE (WHO)/kg (rel. to 88 % TS)

** in % OS
*** in % DM

The distribution of PCDD/F congeners in soil (identical to distribution in grass and grass silage) showed that mainly higher chlorinated dioxins and furans were present.

Before the dairy cows (part 1 of the trial) began grazing on the investigation area, the dioxin content of the milk was very low. After four weeks of grazing the concentrations reached an average value >3 pg WHO PCDD/F-TEQ /g fat, indicating a higher dioxin intake due to the dioxin contaminated grass as well as the dioxin loaded soil. The dioxin concentration decreased at week 8 (probably due to the fact, that the temperature in week four to eight was very high, therefore the feed intake of the cows was comparatively low). At week 10, the dioxin concentrations reached peak values (Table 2). In the following housing period (feeding unloaded feedstuffs) the dioxin level decreased within one week to an average concentration of 2,6 pg pg WHO PCDD/F-TEQ /g fat, after four weeks to 1,23 pg WHO PCDD/F-TEQ /g fat and after eight weeks the concentration in a pooled sample had almost reached the starting value at the beginning of the trial (now 0,62 pg WHO PCDD/F-TEQ /g fat).

Table 2: Dioxin concentration in milk samples during grazing period (part 1)

	time				
	week 0	**week 1**	**week 4**	**week 8**	**week 10**
	Dioxin concentration*				
cow 11	0.33		2.96		4.27
cow 12	0.3		3.13		4.95
cow 13	0.3		5.05		6.33
pooled sample		1.32	3.39	2.36	

*in pg WHO PCDD/F-TEQ /g fat, maximum level reg. Council Regulation (EEC) 2375/2001: 3 pg WHO PCDD/F-TEQ /g fat

At the end of the grazing period large amounts of PCDD/Fs were detected in faeces (Table 3).

Table 3: Dioxin concentration in feces samples at the end of grazing and housing period (part 1)

	time	
	end of grazing period	**end of housing period**
	Dioxin concentration*	
cow 11	6.71	0.19
cow 12	6.4	0.27
cow 13	8.19	0.19

*in ng WHO PCDD/F-TEQ /kg (rel. to 88% DM)

After ten weeks lasting housing period (feeding unloaded feedstuffs) cows were slaughtered and samples of muscle, liver and fat tissues were tested on their dioxin level (Table 4).

Tab. 4: Dioxin concentration in organ/tissue samples after housing period (part 1)

Dioxin concentration *		
liver	**muscle**	**fat tissue**
1.17 +/- 0,057	0.46 +/- 0,08	0.34 +/- 0,106

*mean +/- SD, in pg WHO PCDD/F-TEQ /g fat,
maximum level reg. Council Regulation (EEC) 2375/2001: 3pg WHO PCDD/F-TEQ /g fat (muscle and fat)
6pg WHO PCDD/F-TEQ /g fat (liver)

Table 5 shows dioxin levels in milk during the experimental period (part 2 oh the trial).

Table 5: Dioxin concentration in milk samples (part 2)

Time	Dioxin concentration*		
	Cow 21	**Cow 22**	**Cow 23**
Before drying off	0.38	0.4	0.43
First milk post parturition (p.p.)	6.13	5.85	3.47
Day 3 p.p.	2.58	2.21	0.95
Day 21 p.p.	1.51	1.46	0.81
Day 40/43/58 p.p.	1.18	1.27	0.84
Day 3 post concentrate reduction	1.03	1.18	0.73
Day 7 post concentrate reduction	0.97	1.18	0.78

*in pg WHO PCDD/F-TEQ /g fat, maximum level reg. Council Regulation (EEC) 2375/2001: 3pg I-TEQ/g fat

Before drying off, dioxin concentration in milk was low. After feeding dioxin loaded grass silage (Table 1) for 25 (cow23), 31 (cow 21) and 34 (cow 22) days before calving, the dioxin level in first milk p.p. (colostrum) had reached a level up to 16 times (cow 21) higher than before begin of trial. Fortunately dioxin concentration decreased within three days of feeding unloaded feedstuffs under maximum level again. Within three weeks after parturition concentration further decreased. The reduction of concentrates did not result in higher dioxin levels in milk (Table 5), although there was a need for fat mobilisation due to the insufficient energy supply. The distribution of PCDD/F congeners in milk (first day p.p.) showed, that mainly hexa- and hepta- chlorinated furans were excreted by milk.

Table 6 shows that in the end of trial dioxin levels in all organs and tissues were under maximum level.

Tab. 6: Dioxin concentration in organ/tissue samples after trial (part 2)

Dioxin concentration *		
liver	**muscle**	**fat tissue**
2.27 +/- 1,3	1.11 +/- 0,057	1.32 +/- 0,0224

*mean +/- SD, in pg WHO PCDD/F-TEQ /g fat,
maximum level reg. Council Regulation (EEC) 2375/2001: 3pg WHO PCDD/F-TEQ /g fat (muscle and fat)
6pg WHO PCDD/F-TEQ /g fat (liver)

Discussion

The results show, that there could be a risk on food quality, if food producing animals are grazing on dioxin loaded areas. Fortunately the dioxin concentration in milk samples decreased within one week feeding unloaded feedstuffs under maximum level (3pg WHO PCDD/F-TEQ /g fat) again. Moreover this field study took place under special conditions: the weather was comparatively hot and dry and for this reason the investigation area and the grass were not in a good condition (up to 43, 1% DM in grass) and cows were probably forced to take in larger amounts of soil. Further cows were fed almost exclusively with grass and due to this fact the milk yield was very low (min. 6 l/d). With adequate feeding, the dioxin intake would decrease and milk yield would increase. This could be a possible management strategy that would minimize the dioxin concentration in milk, when dairy cows are grazing on areas, known for a higher dioxin level of the soil.

Further there is an effect on dioxin level in milk after parturition, when dairy cows are fed with dioxin loaded grass silage in the dry period. The dioxin level in the first milk sample after parturition was 16times higher than before drying off and feeding the loaded grass silage. But three days p.p. the level had reached a value under 3pg WHO PCDD/F-TEQ /g fat (maximum level). German milk legislation prescribes, that milk (colostrum) of five days p.p. is not allowed to be sold.
Consequently in this trial milk's dioxin concentration would have been legal at that time, the milk was allowed to be sold again. As a result areas known for their higher dioxin level of soil can be used agriculturally for feeding dry cows, in this case without a risk to the consumer. This has also been proved by the fact the organ and tissue samples had a low level of dioxin. However, the consequences of dioxin accumulation in calves should also be tested to ensure consumer protection.

References

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