DETERMINATION OF FUMONISIN B_1 CONTENT OF PORCINE TISSUES AFTER FEEDING DIET OF HIGH TOXIN CONCENTRATION FOR THE SAKE OF RISK ASSESSMENT

Kovács Melinda[1], Fodor Judit[1], Karsten Meyer[2], Katrin Mohr[2], Johann Bauer[2], Repa Imre[1], Vetési Ferenc[3], Horn Péter[1], Kovács Ferenc[1]

[1] University of Kaposvár Faculty of Animal Science, Research group of the Hungarian Academy of Sciences, Kaposvár, Guba S. u. 40., Hungary
[2] Technical University of Munich, Institute of Animal Hygiene, Freising-Weihenstephan, Germany
[3] Szent István University Veterinary Faculty, Budapest, István u. 2., Hungary

Abstract
The residues deriving from the uptake fumonisin B_1 (FB_1) were determined in growing pigs fed 100 mg/animal (7,5 -7,6 mg/kg bw) daily FB_1 for 5-10 days. The average total FB_1 intake in the first 5 days was 403,8 (365,8-465,8) mg, the daily toxin intake was 30,4-35,9 mg/kg b.w. Among the haematological parameters examined, elevated red blood cell count (9,3-10,8 $x10^6$ /μL), haemoglobin concentration (15,8-17,2 g/dL), haematocrite value (54-66 %) and decreased MCH value (14,3-16,6 pg) were observed. Among the clinical chemical parameters examined the high aspartate aminotransferase activity (116-330 U/L) revealed to hepatic injury. Particular high levels of FB_1 could be measured in kidney (81,6-4762,4 ng/g), liver (73,6-709,6 ng/g), lung (6,4-1144,8 ng/g), spleen (28-7975,2 ng/g) and pancreas (24-464 ng/g). Muscle and fat samples showed negligible contamination (43 and 6 ng/g, respectively). Considering the highest levels in edible tissues (liver, kidney, muscle and fat) and the TDI value recommended by SCF (2000) the consumers risk through a carry-over from swine seems to be negligible.

Key words: fumonisin B_1, porcine tissues, risk assessment

Introduction

The six distinct types of fumonisins known today are produced by the fungi *Fusarium verticillioides*. From the point of view of human exposure, special attention should be paid to FB_1, which is responsible for the development of primary hepatic cancer (Ueno et al, 1997) and oesophageal cancer (Marasas et al., 1988) in humans, encephalomalacia in horses (Marasas et al., 1988) pulmonary oedema in pigs (Fazekas et al., 1998), hepatic cancer in rats and renotoxic and neurotoxic effects in several species of animals.
The chemical structure of fumonisins is very similar to that of sphingolipids, therefore e.g. FB_1 can inhibit the biosynthesis of sphingolipids through blocking the action of the enzyme sphinganine-N-acyltransferase. Changes appearing in the functions and morphology of cells can be attributed partly to the lack of sphingolipids and partly to the accumulation of cytotoxic metabolites such as sphinganine.
The primary source of FB_1 toxicosis is maize. Today it is already generally accepted that the consumption of maize containing high levels of FB_1 is responsible for the development of oesophageal cancer (OC) in humans. It can usually be observed in socially and economically backward regions (South Africa, China, Iran, Northeast Italy, Kenya, Brazil) where the base of the food supply generally consists of inappropriately stored, contaminated and mouldy maize and various foods and drinks (such as beer) made from it.

Analysis of numerous other food items and raw materials in several countries has revealed that they do not constitute any health risk from the point of view of toxin exposure. Thus, the toxin could only be detected from beef only after prolonged exposure of cattle to extremely high doses of FB_1 (Smith and Thakur, 1996). In the United States, FB_1 was detected in only one out of 165 milk samples, in a concentration of >5 ng/ml. Accumulation of the toxin could not be demonstrated in the meat but the toxin was found to accumulate in the liver and kidneys (Prelusky et al., 1994). In laying hens, the toxin does not constitute any significant amounts of residue either in the different tissues or in the eggs.
In order to determine residue formation of fumonisin B_1 in porcine tissues and human health risk arising from this, the short-time effect of FB_1 fed in high dose was examined in weaned pigs.

Materials and methods

Twenty weaned castrated pigs of identical genotype and of approximately 12-14 kg body weight were used. The piglets were divided into two groups (experimental group, n=14 and controls, n=6). They were fed twice a day, and the quantity of any feed not consumed was weighed and recorded. Drinking water was provided *ad libitum*.
The animals were fed a basal diet corresponding to their age (187 g/kg CP, 12.8 MJ/kg ME and 13.1 g/kg LYS content).
After a 5-day adaptation period, a *Fusarium verticillioides* fungal culture containing a known amount of FB_1 was added to the experimental animals' diet to ensure daily fumonisin B_1 intake of 100 mg per animal. The fungal culture was produced in the Veterinary Institute of Debrecen according to the method of Fazekas *et al.* (1998). The control animals were fed a toxin-free diet (T-2, zearalenone, deoxynivalenol and ochratoxin A toxins were not detectable in the diet). Mycotoxin content of the diet was determined by HPLC-system using fluorescence detection, according to the method of Fazekas *et al*. (1996).
Animals were weighed on the 1st, 5th and 11th day of the experiment; their clinical status was continuously monitored. From the blood samples taken on the 5th day standard haematological and biochemical parameters were measured. On days 6 and 11 computer tomographic (CT) examinations were carried out in order to follow the newly developing or progressive changes to the lungs.
On the 6th day of the experiment six experimental animals were exsaunguinated after tranquillisation (Vetranquil 1% inj., Phylaxia-Sanofi, Budapest) and necropsied. The following samples were taken from the organs to determine the FB_1 content: lung, liver, bile, kidney, brain, spleen, pancreas, heart, muscle (*m. longissimus dorsi*, *m. biceps femoris, m. psoas major*), subcutaneous and abdominal fat. At the end of the experiment, on the 11th day, the two still living animals were slaughtered and the same sampling procedure was carried out. Fumonisin B1 content of the organs was determined by by LC-MS according the the method of Meyer et al. (2003).

Results and Discussion

During the feeding experiment one and two pigs died on the 5th and 6th day of the experiment, respectively. Two more animals died on the 8th day. The average total FB_1 intake in the first 5 days was 403,8 (365,8-465,8) mg, the daily toxin intake was 30,4-35,9 mg/kg b.w. (Table 1).
Animals fed 100 mg FB_1 per day became depressed, lost appetite, and their feed intake decreased on the 5th-6 th day. They showed severe dyspnoe, the mucous membranes showed signs of cyanosis. Clinical symptoms developed rapidly and pulmonary oedeme led to death

within 12-24 hours after the first signs. CT examinations also revealed severe pulmonary oedema (Photo 1).

Table 1: Daily and total FB_1 intake

No. of animals	Daily toxin uptake (mg/animal) Experimental day										Total FB_1 uptake
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
1	49,2	65,8	100	100	30,3	39,2	0	*			384,5
2	100	65,8	100	100	100	20,4	30,3	40	100	69,6	726,1
4	100	65,8	100	100	0	*					365,8
5	100	65,8	100	100	100	**					465,8
6	0	65,8	100	100	100	**					365,8
12	0	65,8	100	100	100	62	5	*			432,8
14	100	65,8	100	100	58,3	**					424,1
15	12,5	65,8	100	100	92,5	**					370,8
16	49,5	65,8	100	73,6	100	**					388,9
17	100	65,8	100	88,7	58,3	70	65,5	40	100	69,6	757,9
18	100	65,8	100	100	*						365,8
19	100	65,8	100	100	27,7	*					393,5
20	100	65,8	100	100	41,8	**					407,6

* died ** slaughtered

Among the haematological parameters examined, elevated red blood cell count (9,3-10,8 $x10^6$ /µl), haemoglobin concentration (15,8-17,2 g/dl), haematocrite value (54-66 %) and decreased MCH value (14,3-16,6 pg) were observed on day 6, 8, and 10. This could presumably be due to a compensatory functioning, i.e. more intensive red blood cell and haemoglobin production. Among the clinical chemical parameters examined the high aspartate aminotransferase activity (116-330 U/l) revealed to hepatic injury.

The thoracic cavity of the pigs contained less or higher amount (15-390 ml) of yellow exudates inclined to coagulation. The animals had severe pulmonary oedema (Photo 2). The lungs were enlarged, compact and less elastic to the touch; they did not collapse when the thorax was opened. The interlobular septa were widened because of the serious oedematous infiltration. The trachea and the bronchi contained a white and frothy substance. In some cases haemorrhagic infiltration of the peribronchial lymph nodes was found.

The analysis of the FB_1 content showed detectable amounts of toxin in all organs examined, though concentrations showed very large variations (Table 2). Particular high levels could be measured in kidney, liver, lung, and spleen. However, the described accumulation of FB_1 in liver and kidney (Prelusky *et al.* 1996a, b) could not be confirmed. Muscle and fat samples showed negligible contamination.

Photo 1: CT image of the lung of a pig showing clinical signs on the 5th day of the experiment (interstital pulmonary oedeme)

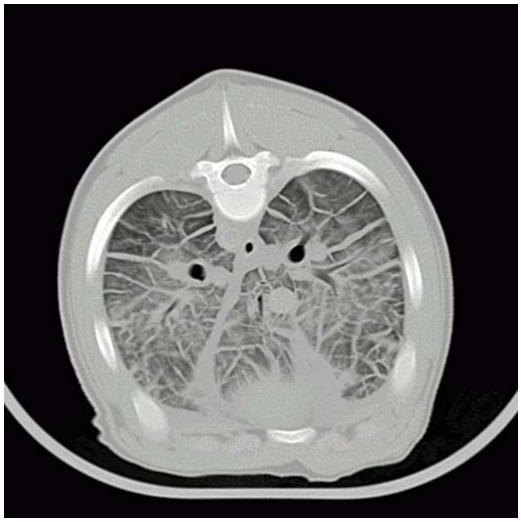
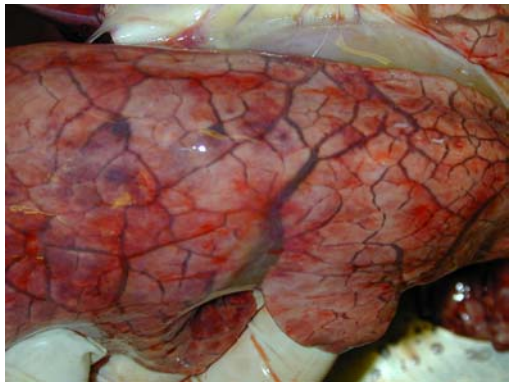


Photo 2: Pathological finding in a pig died on the 8th day of the experiment (interstitial and subpleural pulmonary oedeme)



Individual differences can be attributed to numerous influences. On the one hand, the cumulative uptake of FB_1 varied between the animals (Table 1). On the other hand, because of the rapid clearance of FB_1 in pigs (Prelusky *et al.* 1996a, b) it is more likely that the tissue concentration is correlated to the mycotoxin uptake directly prior to the death. This relationship could be ascertained in the presented study. So the animals with a daily dose of 100 mg FB_1 till death had explicit higher residues in the tissues than the pigs fed no or little mycotoxin the day before exitus. Variations in the individual ability of absorption and metabolism may also have contributed to the individual differences in extractable residues.

Table 2: FB1 content of different organs

Organ	Average FB_1 content* (µg/kg)	Ranges (µg/kg)
Lung	204	6 - 1150
Liver	379	74 - 710
Kidney	1520	82 - 4760
Muscle[1]	43	Nd - 256
Heart	111	3 - 838
Spleen	1020	26 - 7980
Brain	161	Nd - 1860
Fat[2]	6	Nd-11

* contamination was calculated by the averaged concentrations over analytical recovery
[1] average of samples from *m. longissimus dorsi*, *m. biceps femoris*, and *m. psoas major*
[2] average of samples from abdominal and subcutaneous fat

Generally, the implications for consumer health by the consumption of meat from fumonisin-exposed swine are considered to be not of public health concern (ASM 2000, SCF 2000).

According to toxicological studies the NOEL (No Observed Effect Level) is 0,2 mg FB_1/kg bw/day, TDI (Tolerable Daily Intake) calculated is 2 µg/kg bw/day, using a safety factor of 1000 (SCF, 2000).

Table 3: Calculation of human health risk

Organ	max. FB_1 content (ng/g)	Daily consumption (g)	Daily FB_1 uptake (µg)
Muscle	256	300	76,8
Liver	709,6	100	80,0
Kidney	4752,4	50	**237,6**
Fat	921,0	50	46,0

TDI = 2 µg/b.w. kg/day (SCF, 2000), TDI=**120 µg/day FB_1** uptake (in case of 60 kg b.w.)

Considering the highest levels in edible tissues (liver, kidney, muscle and fat) the consumers risk through a carry-over from swine seems to be negligible (Table 3). However, in single cases the impact by the ingestion of heavily contaminated organs may reach toxicological relevance.

References

ASM (American Society of Microbiology), 2000, Comments on Draft Guidance for Industry: Fumonisin Levels in human foods and animal feeds. http://www.asmusa.org/pasrc/fumonisincomments.htm

Fazekas, B., Bajmocy, E., Glavits, R., Fenyvesi, A., and Tanyi, J., 1998, Fumonisin B1 contamination of maize and experimental acute fumonisin toxicosis in pigs. *Journal of Veterinary Medicine B*, **45**, 171-181.

Fazekas, B., Kis, M., and Tóth-Hajdu, E., 1996, Data on the contamination of maize with fumonisin B1 and other fusariotoxins in Hungary. *Acta Veterinarica Hungarica*, **44**, 25-37.

Marasas, W.F., Kellerman, T.S., Gelderblom, W.C., Coetzer, J.A., Thiel, P.G., and van der Lugt, J.J., 1988, Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from Fusarium moniliforme. *Onderstepoort Journal of Veterinary Research*, **55**, 197-203.

Meyer, K., Mohr, K., Bauer, J., Horn, P., Kovács, M.: Residue formation of fumonisin B_1 in porcine tissue. Food Additives and Contaminants, 2003. 20. (7) 639-647.

Prelusky, D.B., Miller, J.D., and Trenholm, H.L., 1996a, Disposition of 14C-derived residues in tissues of pigs fed radiolabelled fumonisin B1. *Food Additives and Contaminants*, **13**, 155-162.

Prelusky, D.B., Trenholm, H.L., Rotter, B.A., Miller, J.D., Savard, M.E., Yeung, J.M., and Scott, P.M., 1996b, Biological fate of fumonisin B1 in food-producing animals. *Advances in Experimental Medicine and Biology*, **392**, 265-278.

Prelusky, D.B., Trenholm, H.L., and Savard, M.E., 1994, Pharmacokinetic fate of 14C-labelled fumonisin B1 in swine. *Natural Toxins*, **2**, 73-80.

SCF (Scientific committee on food), 2000, Opinion of the scientific committee on food on fusarium toxins - part 3: Fumonisin B_1 (FB_1), expressed on 17 October 2000. http://europa.eu.int/comm/food/fs/sc/scf/out73_en.pdf

Smith, J. S., Thakur, R. A. 1996. Occurrence and fate of fumonisins in beef. Advances in Experimental Medicine and Biology **392**:39-55.

Ueno, Y., Lijima, K., Wang, S-D., Sugiura Y., Sekijima, M., Tanaka T., Chen, C., Yu. S-Z. (1997). Fumonisins as a possible contributory risk factor for primary liver cancer: A 3-year study of corn harvested in Haimen, China, by HPLC and ELISA. Food and Chemical Toxicology, **35**. 1143-1150.