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The distribution and elimination of fumonisin analogues in weaned piglets after oral administration of *Fusarium verticillioides* fungal culture

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Methods/Techniques:	Toxicology - animal study
Additives/Contaminants:	Fumonisin
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Table 1. Clinicochemical variables of the piglets included in the study.

	Day 1	Day 7	Day 14	Day 21
ALT (U l ⁻¹)	*	**	**	*
Control	44±4	50±8.8	72±13.5	61±21.5
Treated	53±9.1	62±12.4	87±16.1	89±17.2
AST (U l ⁻¹)				
Control	25±3.5	37±14	42±15.3	35±15.5
Treated	26±7.1	34±9.5	38±13.8	46±15.6
CREA (mmol l ⁻¹)				
Control	67±8.7	86±9	80±12.1	88±12.1
Treated	68±7.6	86±9.6	92±13	91±11.7
ALP (U l ⁻¹)				*
Control	643±148.1	609±86	530±130.2	379±154.0
Treated	699±60.4	647±44.5	674±161.7	639±98.5

* P ≤ 0.05; ** P ≤ 0.08

Table 2. Absolute quantity of fumonisins taken up during the 22-day period of study and total FB₁ uptake per kg of body weight.

Animal no.	270	271	272	273	274	275	276	277	278	279	Mean	±SD ^a
Final body weight (kg)	20.1	22.8	21.0	22.4	21.8	20.2	19.8	19.6	23.5	20.3	21.15	1.4
Total FB ₁ uptake (mg)	1100	1100	1100	1100	1080.5	1066.5	1100	1065.8	1100	1100	1091.2	14.5
Total FB ₂ uptake (mg)	415.0	415.0	415.0	415.0	407.7	402.5	415.0	402.2	415.0	415.0	411.7	5.4
Total FB ₃ uptake (mg)	113.3	113.3	113.3	113.3	111.4	109.9	113.3	109.9	113.3	113.3	112.4	1.45
Total FB ₁ uptake per kg of body weight (mg kg ⁻¹ of final body weight)	54.7	48.2	52.3	49.1	49.5	52.7	55.5	54.3	46.8	54.1	51.7	3.0

^a Standard deviation

Table 3. Concentrations of fumonisin B₁^a and fumonisin B₂^a in tissues following oral administration^b to pigs

No. of animals ^c	Lung		Liver		Kidney		Spleen		Myocardium		M. longissimus dorsi		M. psoas major		Abdominal fat		Subcutaneous fat	
	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂
270	4	1.6	117.6	ND	47.2	ND	ND	ND	ND	ND	1.6	ND	0.8	ND	1.6	1.6	2.4	ND
271	1.6	2.4	104.8	ND	25.6	ND	4.8	ND	ND	ND	ND	ND	ND	ND	1.6	ND	0.8	3.2
272	1.6	ND ^d	116	ND	36.8	ND	11.2	ND	ND	ND	ND	ND	0.8	ND	1.6	ND	ND	1.6
273	1.6	ND	77.6	ND	14.4	ND	8.8	ND	8	ND	ND	ND	ND	ND	0.8	ND	0.8	ND
274	2.4	0.8	70.4	ND	30.4	ND	6.4	ND	10.4	ND	1.2	ND	ND	ND	2.4	2.4	ND	0.8
275	3.2	ND	120.8	7.2	36	ND	31.2	ND	8.8	ND	ND	ND	ND	ND	8.8	3.2	2.4	ND
276	2.4	ND	133.6	0.8	26.4	ND	ND	ND	12.8	ND	ND	ND	ND	1.6	17.6	3.2	8.8	1.6
277	3.2	ND	158.4	3.2	43.2	ND	5.6	ND	7.2	ND	1.6	ND	6	ND	111.2	16	3.2	1.6
278	3.2	ND	56	2.4	23.2	ND	3.2	ND	35.2	ND	ND	ND	1.2	ND	ND	ND	7.2	2.4
279	5.6	1.6	38.4	0.8	22.4	ND	2.4	ND	10.4	ND	ND	ND	ND	ND	0.8	ND	1.6	0.8
Mean	2.8	0.6	99.4	1.4	30.6	-	7.4	-	9.2	-	0.4	-	0.8	0.2	14.6	2.6	2.72	1.2
±SD ^e	±1.2	±0.9	±37.5	±2.3	±10.1	-	±9.1	-	±10.2	-	±0.7	-	±1.8	±0.5	±34.3	±4.8	±2.9	±1.0

^a ng g⁻¹.^b FB₁-B₂-B₃ impact is shown in table 2.^c Pigs nos 265-269 were used as controls. In these samples, fumonisin B₁ and B₂ could not be detected.^d Not detected. LOD for liver, lung, kidney, muscle, myocardium: 5 µg kg⁻¹
LOD for muscle, fat, brain spleen: 2 µg kg⁻¹.^e Standard deviation

Table 4. Excretion of **fumonisin** in the faeces and urine
between days 13 and 17 of the experiment

Animal no.	Excreted in urine (mg)		Excreted in urine (%)		Excreted in faeces (mg)		Excreted in faeces (%)		Total excretion (mg)		Total excretion (%)	
	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂	FB ₁	FB ₂
270	0.9	0.005	0.4	0.05	34.4	2.3	13.7	2.4	35.3	2.35	14.1	2.4
271	1	0.1	0.4	0.1	21.8	3.5	8.7	3.7	22.8	3.6	9.1	3.8
272	1.9	0.2	0.8	0.2	14.5	1.9	5.7	2	16.4	2.1	6.5	2.2
273	3.6	0.2	1.4	0.2	63.5	2.7	25.4	2.8	67.1	2.9	26.8	3
274	9.7	1	3.8	1	60.4	3.5	24.2	3.7	70.1	4.5	28	4.7
275	0.9	0.1	0.4	0.1	2.4	0.9	0.9	0.9	3.3	1	1.3	1
276	9	1	3.6	1	2.3	0.4	0.9	0.4	11.3	1.4	4.5	1.5
277	4.4	0.4	1.7	0.4	76.1	2.6	30.5	2.7	80.5	3	32.2	3.1
278	1.9	0.2	0.8	0.2	0.3	0.4	0.1	0.4	2.2	0.6	0.9	0.6
279	11.5	1.6	4.6	1.7	6.4	2	2.5	2.1	17.9	3.6	7.1	3.8
Mean	4.5	0.5	1.8	0.5	28.2	2	11.3	2.1	32.7	2.5	13.1	2.6
±SD	±3.9	±0.5	±1.5	±0.5	±27.3	±1.1	±10.9	±1.2	±27.8	±1.3	±11.1	±1.3

Note: The total uptake of FB₁, FB₂ and FB₃ (250, 94.3 and 25.7 mg, respectively) was considered as 100 %.

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Figure 1. a. FB₁ precense in different tissues (box-whisker plot^a)

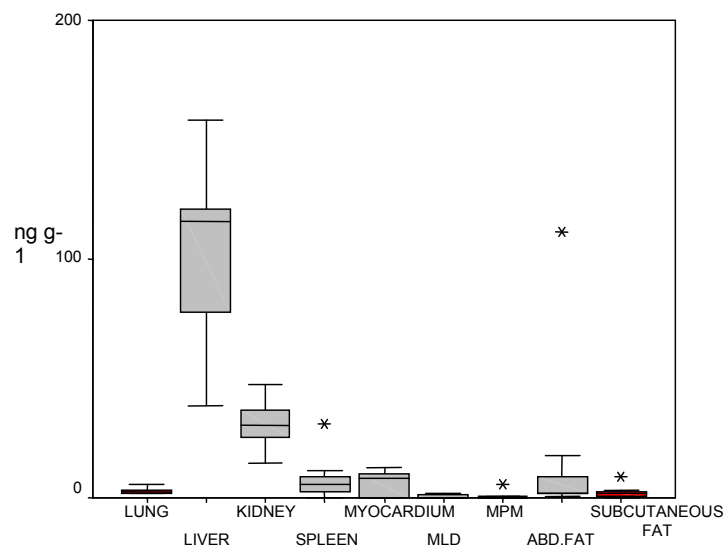
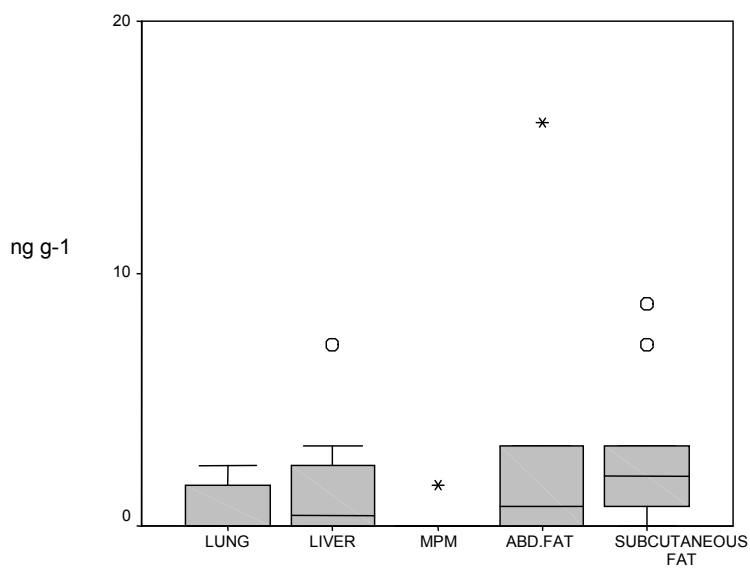
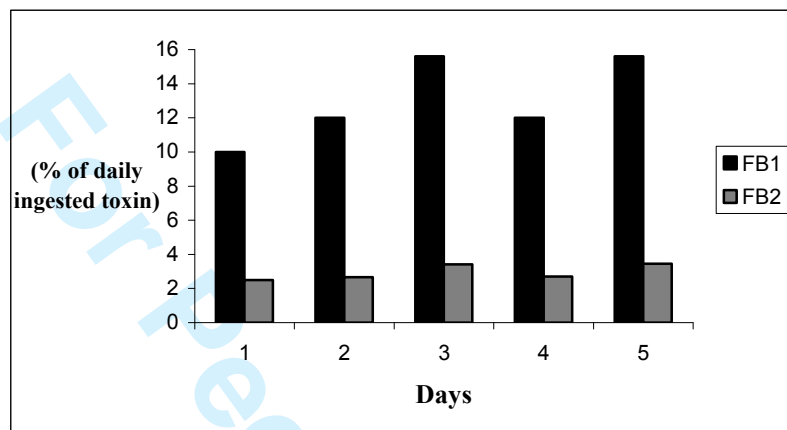


Figure 1 b. FB₂ precense in different tissues (box-whisker plot^a)



^a The box has lines at the lower quartile (25%), median, and upper quartile (75%) values. The whiskers are lines to the extreme values.

Figure 2. Averaged excretion of **fumonisin**s (in percent of daily ingested toxin) during the 5 days.



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The distribution and elimination of fumonisin analogues in weaned piglets after oral administration of *Fusarium verticillioides* fungal culture

The distribution and elimination of fumonisins after oral administration of 50 mg FB₁, 20 mg FB₂ and 5 mg FB₃/animal/day for 22 days was studied in weaned barrows. At the end of the trial, the lung, heart, liver, kidney, spleen, brain, pancreas, serum, bile, muscle, fat, urine and faeces samples were collected and their content of fumonisins (FB₁, FB₂ and FB₃) was determined by LC-MS. The highest FB₁ concentrations were found in the liver ($99.4 \pm 37.5 \text{ ng g}^{-1}$) and kidneys ($30.6 \pm 10.1 \text{ ng g}^{-1}$), whilst the highest average amount of FB₂ was found in the liver ($1.4 \pm 2.3 \text{ ng g}^{-1}$) and fat ($2.6 \text{ ng g}^{-1} \pm 4.8$) samples. Comparing the FB₁/FB₂ ratio in different organs (19/1) it was found that the ratio in the abdominal and subcutaneous fat samples (4/1) was markedly different from those in all other tissues, namely the relative proportion of FB₂ was higher in latter cases. Of the total quantity of FB₁ 13% taken up during 5 days was excreted unchanged with faeces and urine. On average, in the urine and in the faeces FB₁ was detected in 9- and 14-fold quantities, as compared to FB₂.

Keywords: fumonisins, pig, residues, elimination, distribution

Deleted: ¶

Introduction

Fumonisin were isolated first in 1988 from a culture of *Fusarium verticillioides* (earlier *Fusarium moniliforme*) strain MRC 826 grown on maize (Gelderblom et al. 1988, Cawood et al. 1991), and subsequently their structure was also determined (Bezuidenhout et al. 1988). Toxins of this group are produced by fungus species belonging to the *Liesola* section of the genus *Fusarium*, most often by *Fusarium verticillioides* and *Fusarium proliferatum* and less frequently by other *Fusarium* species related to the former.

Fumonisin are regarded as potentially one of the most dangerous mycotoxins (group 2B) with respect to carcinogenicity in humans (IARC 1993). Fumonisin B₁ (FB₁) causes leukoencephalomalacia in horses (Marasas et al. 1988) and pulmonary oedema in swine (Harrison et al. 1990). It is carcinogenic and has been implicated in the pathogenesis of oesophageal cancer in humans (Marasas et al. 1988, 1993). Fumonisin B₁ is nephrotoxic and hepatotoxic in rabbits (Gumprecht et al. 1995), and it has also been shown to exert deleterious effects on the haematopoietic organs (Mariscal-Quintanar et al. 1997).

While earlier investigations into the effects of this toxin were prompted by animal health considerations, by now the focus of interest has shifted to the human food safety implications. The exact mechanism of action of FB₁ has still not been elucidated. Fumonisin are very similar to sphingolipids in molecular structure (Shier 1992); thus, they interfere in the metabolism of the latter and disturb the processes mediated by these molecules (Kim et al. 1991).

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3 Maize is the primary source of FB₁ toxicosis. The results of surveys indicate that
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5 fumonisins contaminate the maize kernels in all corn-growing countries of the world
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7 and can cause fumonisin toxicosis (Dutton 1996). These mycotoxins can even be
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9 present in apparently healthy maize. Maize and maize-based products are most widely
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11 used for human consumption in Africa and in some Asian countries, but in smaller
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13 quantities maize-based products are consumed by the human population in numerous
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15 countries of the world (Shephard et al. 1996).
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19 Surveys and testing projects conducted between 1992 and 1997 have demonstrated
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21 fumonisin contamination in most of the European maize-growing countries (Visconti et
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23 al. 1995, Shephard et al. 1996). Maize and maize-based products for human
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25 consumption contain fumonisins even in the highly developed countries such as the
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27 USA, Italy and France (Sydenham et al. 1991, Stack and Eppley 1992, Bullerman and
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29 Tsai 1994, Doko and Visconti 1994, Visconti et al. 1995, Bullerman 1996).
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33 The fumonisin B analogues, including toxicologically important FB₁, FB₂ and FB₃ are
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35 the most abundant naturally occurring fumonisins (Marasas, 1996). FB₁ typically
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37 accounts for 70 to 80 % of the total fumonisins produced, while FB₂ usually makes up
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39 15 to 25 % and FB₃ usually from 3 to 8 %, when cultured on corn or rice (Branham and
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41 Plattner, 1993; Marín et al., 1995). Food materials of animal origin may become
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43 contaminated with FB₁ after the toxin has been absorbed from the digestive tract,
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45 entered the bloodstream and reached the peripheral tissues. Therefore, from the food
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47 safety point of view it is especially important to know the distribution of this mycotoxin
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3 in the organism. As opposed to the 50–65% bioavailability of deoxynivalenol (DON) or
4 ochratoxin A, the bioavailability of FB₁ is as low as 0–6% (Galtier 1998).
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9 Numerous experiments have been conducted on various animal species in several
10 countries to study the possible FB₁ contamination of foods of animal origin. It appears
11 that foods of animal origin do not pose the risk of fumonisin B₁ exposure to humans.
12 Thus in beef, FB₁ could be detected only after prolonged exposure of cattle to extremely
13 high doses of the toxin (Smith and Thakur 1996). In the USA, FB₁ was detected in a
14 concentration exceeding 5 ng ml⁻¹ in only one out of 165 milk samples tested (Richard
15 et al. 1996). The toxin could not be detected in sow's milk even after feeding a diet
16 containing 100 mg kg⁻¹ FB₁ to pigs for a period of two weeks (Becker et al. 1995). In a
17 study conducted in Hungary in which a fungal culture containing high doses of
18 fumonisin B₁ (100 mg/day/animal) was fed to pigs, the toxin was detectable in sow's
19 milk in concentrations of 18.0–27.5 ng g⁻¹ (Zomborszky-Kovács et al. 2000).
20 Accumulation of the toxin could be demonstrated in the liver and kidney, but not in the
21 meat, of pigs (Prelusky et al. 1994). Fumonisin B₁ does not form substantial amounts of
22 residues either in the different tissues or in the eggs of laying hens (Vudathala et al.
23 1994).
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41 In order to detect fumonisin B₁ appearing in food raw materials of animal origin, in an
42 earlier experiment (Meyer et al. 2003) we studied the effects of relatively short (5- and
43 10-day) exposure to high doses of FB₁ (100 mg/day/animal) in weaned piglets. There
44 was no statistically significant correlation between the total quantity of toxin appearing
45 in the organs of animals and the amount of toxin ingested. Although the distribution of
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3 the toxin in the different organs showed great individual variation, it could be
4 established that the highest amounts of toxin were detectable in the spleen (maximal
5 concentration: 7980 ng g⁻¹) and kidney (maximal concentration: 4760 ng g⁻¹), while in
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7 the liver FB₁ was present in concentrations of 73.7–709.6 ng g⁻¹. In some cases,
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9 substantial concentrations of FB₁ could be measured in the lungs and pancreas as well.
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11 Samples taken from muscle and adipose tissue, which are principally used for human
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13 consumption, did not contain fumonisin B₁ in appreciable amounts. According to the
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15 results of model calculations made with the maximal tissue concentrations of FB₁
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17 measured in the experiment, humans do not ingest notable amounts of FB₁ with food
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19 raw materials originating from pigs.
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25 However, high doses resulting in acute toxicosis possibly influence the metabolism and
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27 pharmacokinetic properties of the toxin. For an actual risk assessment, we considered it
28
29 necessary to study the effects of prolonged exposure to lower doses of the toxin. Since
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31 all main analogues (FB₁, FB₂ and, to a lesser extent, FB₃) occur at significant levels in
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33 contaminated maize generally and that FB₂ and FB₃, like FB₁, act as inhibitors of the
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35 enzyme sphingosine N-acyltransferase, exact information on all analogues' distribution
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37 in the organism is necessary.
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41 In this experiment, the distribution and detectability of fumonisin analogues in the
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43 different organs and the elimination of those from the organism were determined after a
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45 22-day exposure to a combined toxin dose of 50 mg FB₁, 20 mg FB₂ and 5 mg
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47 FB₃/animal/day.
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Materials and methods

Experimental animals, design of the experiment

Fifteen weaned barrows of the same genotype, weighing 12–14 kg, were used in the experiment. The piglets were weighed and then divided into two groups: an experimental group (n = 10) and a control group (n = 5). The animals were placed in metabolic cages during the trial. The temperature (20°C) of the trial room was controlled in accordance with the needs of weaned piglets. Feed was given twice a day, in two equal portions, and the amount of feed not consumed by the animals was measured. Drinking water was available *ad libitum* via automatic drinkers. The duration of the trial was 22 days (following a 5-day adaptation phase), during which time the animals were weighed weekly. Prior to the feeding of toxins, blood samples were taken from the piglets and the baseline haematological and clinicochemical parameters were determined. After the experimental feeding had been started, blood samples were taken from the animals weekly.

At the end of the trial, the piglets were killed by bleeding after sedation. Gross pathological examination was performed and the lungs, heart, liver, kidneys, spleen, brain and pancreas were weighed. The trial was approved by the Animal Experimentation Ethics Committee of the University of Kaposvár (KÁ-16/2001).

Feeding

The experimental animals were fed a basic ration of a composition corresponding to their age (187 g kg⁻¹ crude protein, 12.8 MJ kg⁻¹ metabolizable energy, 13.1 g kg⁻¹ lysine). After the 5-day adaptation period, a *Fusarium verticillioides* fungal culture was

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3 mixed into the ration of the experimental animals, so as to provide a daily toxin intake
4 of 50 mg FB₁, 20 mg FB₂ and 5 mg FB₃/animal. The fungal culture was mixed into the
5 ration freshly every day, in a half-dose for each of the two feedings.
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10 11 *The production of fumonisins*

12 Fumonisins were produced at the Veterinary Institute of Debrecen using a locally
13 developed method (Fazekas 1998), as discribed below. The *F. verticillioides* strain
14 designated 14/A was isolated from a maize sample containing a high fumonisin B₁
15 concentration (75 mg kg⁻¹) and was identified morphologically by methods described in
16 mycological manuals (Samson and Van Reenen-Hoekstra, 1988). The toxin was
17 produced experimentally on maize by the following way:
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25 Maize with moisture content of 32% was autoclaved during two consecutive days and
26 was inoculated with the 7-day-cultivated *F. verticillioides* strain. The culture was
27 incubated in darkness at 24°C for 3 weeks. After opening, the fungus-infected maize
28 was dried at room temperature for some days, then ground. The homogenized fungal
29 culture contained FB₁, FB₂ and FB₃ at a concentration of 7300 mg kg⁻¹, 2750 mg kg⁻¹
30 and 750 mg kg⁻¹, respectively.
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39 *Determination of mycotoxin content in feeds and fungal cultures*

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42 The mycotoxin content of control and experimental feed was checked. T-2 toxin was
43 determined with GC/MS (LOD: 0.01 mg kg⁻¹), while zearalenone (LOD: 0.01 mg kg⁻¹),
44 DON (LOD: 0.05 mg kg⁻¹) and ochratoxin A (LOD: 0.1 µg kg⁻¹) by an HPLC system.
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47 The determination of FB₁, FB₂ and FB₃ content from the culture and feed samples was
48 determined also by a HPLC fluorescence detection method based upon derivatisation
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3 with o-phthaldialdehyde (OPA) (Fazekas et al., 1997; Fazekas, 1998), as described
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9 For the assay of feed samples 25 g of sample, while for the production of fumonisins
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11 2.5 g of fungal culture was extracted with 100 ml of a 3:1 mixture of methanol and
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13 water. A 10 ml volume of the filtered extract was purified on a SAX (strong anion
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15 exchange) SPE (solid phase extraction) column (Varian). Fumonisins were eluted from
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17 the SAX column with methanol containing 1 % acetic acid. The evaporated residue was
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19 dissolved in methanol (in 0.2 ml of methanol when assaying food samples, and in 1 ml
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21 of methanol when analysing fungal cultures), and the solution was then further diluted
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23 10- to 50-fold depending on the mycotoxin content.
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27 The derivative from with OPA and mercaptoethanol was separated on a 250 x 4 mm
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29 LiChrospher RP 18 column of 5 µm particle size, using a 50:50:1 eluent mixture of
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31 acetonitrile, water and acetic acid as eluent. Detection was by a HP 1046 type
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33 fluorescence detector (excitation and emission wavelengths 335 and 440 nm,
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35 respectively). The LOD values for the different toxins samples were: FB₁: 0.05 mg kg⁻¹;
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37 FB₂: 0.1 mg kg⁻¹; FB₃: 0.1 mg kg⁻¹.
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41 The diet fed to the control group did not contain detectable amounts of mycotoxin.
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44 45 *Study of toxin excretion*

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47 In order to monitor the excretion of fumonisins, in the third week of the experiment
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49 (between days 13 and 17 of the toxin feeding period) the total quantity of urine and
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3 faeces was collected over a period of 5 days. Faeces was collected twice daily
4 (following the morning and afternoon feeding), and weighed with gram precision. Every
5 day the entire quantity of faeces was homogenised and, after the determination of dry
6 matter content, 30% of the total quantity was lyophilised, ground and prepared for
7 laboratory analysis.
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15 Urine was collected continuously into a sealed container connected, and its volume was
16 measured following the morning feeding. The urine collected daily was homogenised
17 and 15% was stored at -18 °C until further processing.
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22 *Haematological and biochemical examinations*

23
24 The blood samples taken during the experiment were subjected to standard
25 haematological examination. This involved the determination of the following
26 variables: red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin
27 concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean
28 corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration
29 (MCHC), and thrombocyte count. The measurements were made with a CELL-DYN
30 3500 type haematological analyser (ABBOTT, Germany). The total protein, albumin,
31 creatinine and bilirubin concentrations as well as aspartate aminotransferase (AST),
32 alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), lactate
33 dehydrogenase (LDH) and alkaline phosphatase (ALP) activities of the blood plasma
34 were also determined with the Vetest 8008 biochemical analyser (IDEXX Cergy
35 Pantoise Cedex, Libourne, France), using Eastman Kodak test plates (Eastman Kodak
36 Company for IDEXX Laboratories, Inc., Maine, USA).
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5 *Determination of the fumonisins content of organs, faeces and urine*
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7 The amount of toxins detectable in different organs and tissues including the liver,
8 lungs, kidney, brain, spleen, heart, the *m. longissimus dorsi* and *m. psoas major*
9 muscles, and abdominal and subcutaneous fat was determined. Furthermore, the toxin
10 content of faecal, urine, and bile samples was measured.
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17 Quantification and identification of fumonisins was carried out using LC-MS by the
18 method of Meyer et al. (2003) with some modifications. Essentially, the extraction of
19 the tissues was performed using microwave-accelerated extraction, resulting in higher
20 precision of the analysis of FB₁ particularly in fatty samples.
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27 Regarding the toxin determination from faeces, lyophilized samples (0.5 g) were
28 extracted by a mixture of 0.1 M ethylenediaminetetraacetate (EDTA) and methanol (8
29 ml; 4:1 v/v) for 30 min. The sample was filtrated through a fibre glass filter (Whatman
30 GF A, Dassel, Germany) and an aliquot of the filtrate was analysed by LC/MS. The
31 mean recovery rates for FB₁ and FB₂ in urine were $98.5 \pm 7.3 \%$ and $98.9 \pm 8.9\%$; the
32 accordant values for faeces were $84.2 \pm 6.8 \%$ and $59.1 \pm 11.2 \%$, respectively; the limit
33 of determination was for both mycotoxins $2 \mu\text{g kg}^{-1}$ in urine and $50 \mu\text{g kg}^{-1}$ in faeces.
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40 Taking the low feed concentrations of FB₃ into account, as compared to the other FB
41 forms, and its presence supposedly under the LOD value, these parameters were not
42 aimed to determine.
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49 *Statistical analysis*
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3 All basic data (means, standard deviations, extreme values) were evaluated. Differences
4
5 between the control and the treated group in haematological and biochemical variables
6
7 and mean organ weights were detected by the two-sample *t*-test on a probability level of
8
9 $P \leq 0.08$. Correlation analysis ($P \leq 0.05$) was performed to determine the correlation
10
11 between between the concentration of FB₁ and FB₂ in the abdominal fat samples .
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15 Data processing and the mathematical-statistical calculations were performed using the
16
17 Compare Means (Independent-Samples-*t*-Test, Paired-Samples-*t*-Test), Correlate and
18
19 Descriptive Statistics modules of the SPSS 10.0 programme package, the SAS System
20
21 (Local, WIN_PRO) programme package, and the spreadsheet and figure editor
22
23 programmes of EXCEL 7.0.
24
25

26 27 **Results and Discussion**

28 29 *Clinical signs*

30
31 The pigs fed the experimental diet did not show clinical signs of disease. Their feed
32
33 consumption was balanced throughout the experiment and no feed refusal indicative of
34
35 toxin effect could be observed. Only a single animal (no. 277) showed a transient
36
37 decrease in feed intake on two occasions.
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40 41 *Haematological and biochemical results*

42
43 The haematological variables of the experimental animals did not differ from the
44
45 physiological values (Friendship and Henry, 1992). The clinical chemical variables
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47 determined varied within the physiological range (Friendship and Henry 1992). As
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49 compared to the values of the control pigs, the ALT activity (at all the four testing
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3 times) of the experimental animals were significantly elevated (Table 1), presumably as
4
5 an indication of the pathological processes taking place in the liver and lungs.
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9 The ALP activity was significantly different between groups at the last sampling,
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11 although this was rather a decrease in the control group and not an increase in the
12
13 treated.
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17 [Insert Table 1 about here]
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20 21 *Findings of the pathomorphological examinations*

22
23 Pulmonary oedema, the typical disease caused by fumonisin contaminated feed,
24
25 developed in all of the piglets as a result of the toxin dose applied. The apical and
26
27 cardiac lobes of the lungs exhibited oedema of varying severity and were consolidated
28
29 and compact to the touch. In the pleural cavity of a few animals a small volume (15–50
30
31 ml) of slightly yellowish fluid with a tendency to clot could be found.
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35 Petechial haemorrhages and extensive erosions (incipient ulcers) were seen on the
36
37 gastric mucosa of all the animals. Of the other organs examined, the liver, the heart,
38
39 and the kidneys showed pathological changes. The most remarkable pathological
40
41 findings included e.g. the enlarged, friable, pale, yellowish liver, the pale kidney with
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43 spot-like necrotic areas, and the small volume of pericardial fluid containing lumps.
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45 There was no statistically significant difference between the two groups in the weight of
46
47 the organs. No changes indicative of toxin effect were found in the control animals.
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3 *Fumonisin content of the organs examined*
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5 Tables 2 and 3 present the FB₁ and FB₂ concentrations of the different organs after the
6
7 22-day period of feeding the toxin-containing diet. The results were evaluated on the
8
9 basis of total quantity of fumonisins taken up during the trial as well as the total
10
11 quantity taken up per kg of body weight.
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15 During the 22-day feeding period, the mean toxin intake of the animals was 1091.2 mg
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17 FB₁, 411.7 mg FB₂ and 112.4 mg FB₃, while the total FB₁ uptake per kg bw was 51.7 ±
18
19 3.0 mg kg⁻¹.
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23 [Insert Table 2 about here]
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25

26
27 The highest concentrations of FB₁ were found in the liver and kidneys. Compared to
28
29 FB₁ content, FB₂ was detected in lower concentrations in these tissues (Figure 1. a, b).
30
31 Relatively high amounts of FB₁ were measured in the myocardium and spleen, while
32
33 markedly lower FB₁ concentrations were measured in the lungs. These findings show
34
35 good agreement with the results obtained in the experiment of Prelusky et al. (1994,
36
37 1996b), according to which the highest toxin levels could be measured in the liver and
38
39 kidneys of pigs fed a diet containing 2–3 mg kg⁻¹ radiolabelled FB₁ for a period of 24
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41 days.
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45 [Insert Figure 1. a and b about here]
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3 The toxin could not be detected in the brain in any of the cases. In a previous study
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5 (Meyer et al. 2003), FB₁ was detectable in the brain of the majority of animals fed the
6
7 toxin in a dose of 100 mg/animal/day. FB₁ was usually present in higher concentrations
8
9 in the brain of animals having died of severe clinical signs of toxicosis than in the brain
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11 of animals slaughtered after having survived the toxin exposure. In the present study,
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13 the pigs were thoroughly exsanguinated at slaughter. The FB₁ content found in the brain
14
15 in the previous study might possibly be attributed to the toxin content of residual blood
16
17 remaining in the brain.
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21 FB₁ could not be detected in the majority of muscle samples, i.e. in the tissue type most
22
23 widely used for human consumption. Nor were there appreciable amounts of FB₁ in the
24
25 adipose tissue samples, with the exception of a single pig, the abdominal fat tissue of
26
27 which contained 111.2 ng FB₁ g⁻¹ of tissue. This could probably be attributed to
28
29 individual sensitivity.
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33 Although in most cases the FB₂ content of the abdominal and subcutaneous fat samples
34
35 was generally negligible, in these tissues the average presence of this analogue was
36
37 higher (4/1) than in the other tissues. This was especially true when comparing the FB₁
38
39 to FB₂ appearance ratio in the different tissues (19/1).
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43 [Insert Table 3 about here]
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47 In the previous experiment (100 mg FB₁/animal/day; Meyer et al. 2003) there was no
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49 statistically significant correlation between the total quantity of toxin appearing in the
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3 organs of animals and the quantity of toxin ingested. In the present experiment a close
4 correlation ($r = 0.997$, $P < 0.05$) was found between the concentration of FB_1 and FB_2 in
5 the abdominal fat samples. .
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11 No detectable levels of fumonisins could be measured in the organs of the control pigs.
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14 15 *Excretion of fumonisins*

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17 According to data of the literature (Norred et al. 1993, Shephard et al. 1994a) and the
18 results of our own study (Meyer et al. 2003), some of the absorbed toxin is secreted
19 with the bile into the alimentary tract, from where it might be excreted with the faeces.
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21 Of the animals used in this study, only pig no. 277 had FB_1 in the bile, but in that
22 animal the toxin was present in the bile in a high concentration (322.8 ng g^{-1}).
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29 The toxin concentration detectable in the organs and urine is influenced also by the
30 metabolism of the toxin. Some of the mycotoxins can be transformed by the intestinal
31 microflora, which affects the toxin content of the faeces. The metabolism of mycotoxins
32 may result in less toxic substances (e.g. in the case of DON) or more toxic metabolites
33 (e.g. in the case of aflatoxin). Only few data have been reported in the literature about
34 the metabolism of FB_1 . In experiments conducted *in vitro* with liver and renal epithelial
35 cells of rats, molecular transformation of FB_1 did not occur (Niel 1998).
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45 In vervet monkeys FB_1 was excreted mainly in faeces as the intact molecule or its
46 partially- or fully-hydrolysed metabolites (Shephard et al., 1994b). As has been
47 demonstrated for FB_1 , most of the gavage dose was recovered in faeces, either as FB_2
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3 itself or as a partially- or fully-hydrolysed moiety. Owing to the low bioavailability of
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5 fumonisins, this hydrolysis must occur in the gut by the action of intestinal esterase
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7 enzymes or the action of microorganisms (Shephard et al., 1999).
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11 [Insert Table 4 about here]
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15 The average FB₁ content of faecal samples collected between days 13 and 17 of the
16
17 experiment was 48.8 µg g⁻¹ per day. Much less FB₁ toxin (on average 304.7 ng ml⁻¹)
18
19 was excreted in the urine daily. Accordingly, during the 50 mg FB₁ day⁻¹ consumption
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21 for 5 days, the total amount of FB₁ excreted by urine was on average 4.5 mg, while by
22
23 faeces 28.2 mg.
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27 On average, 13% of FB₁ quantity taken up during 5 days was excreted with the faeces
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29 and urine, about 86% with the faeces and only 14% with the urine (Table 4). From FB₂
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31 taken up during this period a strongly lower average quantity (in the urine and faeces
32
33 0.5 % and 2.1 %, respectively) was detected in these excreta. These findings show good
34
35 agreement with the results obtained in the experiment of Shephard et al. (1999),
36
37 according to which 0.2 % of the single gavage FB₂ dose to vervet monkeys was
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39 recovered in urine over a 7-day period. The predominant route of excretion was via the
40
41 faeces, mainly as the unmetabolized toxin (8 % in the 7-day period) or as a partially
42
43 hydrolysed analogue. The fate of the remainder of the dose was unknown (Shephard et
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45 al., 1999).
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3 Generally, in the urine and in the faeces the FB₁ analogue was detected in 9- and 14-
4 fold quantities, as compared to FB₂. In the feed the FB₁-FB₂ ratio was 2.6:1, while the
5 total FB₁-FB₂ excretion ratio was 13:1. Consequently, the elimination of FB₂ was less
6 than that of FB₁. There was no statistically significant correlation between the total
7 quantity of FB₁ excreted in the urine and that excreted in the faeces.
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15 The average amount of FB₁ excreted daily increased steadily in the first three days of
16 the period examined, then on day 4 it decreased by 24% as compared to the preceding
17 day (Figure 2).
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23 [Insert Figure 2 about here]
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27 In the experiment of Prelusky et al. (1996b), in which FB₁ mycotoxin was fed to rats in
28 daily doses of 2–3 mg kg⁻¹ over a period of 33 days, in the first 3 days of mycotoxin
29 loading <35% of the total dose was excreted with the urine and faeces, then from day 9
30 onwards 70% of the daily dose was eliminated per day on average. In that study, the use
31 of radiolabelled FB₁ enabled the researchers to detect also the metabolised chemical
32 forms of FB₁ in the excretions. According to certain data of the literature, 60–90% of
33 the total toxin quantity appearing in the faeces of cows occurs in hydrolysed form
34 (Prelusky et al. 1995).
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45 The results of the present experiment refer only to the unchanged chemical form of FB₁
46 only. The metabolism of fumonisins in the gut could be a possible explanation of the
47 loss of the remainder. As in the study with rats, the majority of ¹⁴C label dosed orally
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3 was recovered in faeces (approximately 90%) with less than 1% recovered in urine
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5 (Prelusky et al. 1994, 1996a).
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9 It is doubtful whether the results obtained in rats can be adapted to pigs. Namely,
10 according to the results of a study the elimination of the toxin and the body weight are
11 in inverse ratio, e.g. from mice and rats the toxin is rapidly eliminated while toxin
12 elimination from humans is supposed to be slower (DeLongchamp and Young 2001).
13 This hypothesis is supported by the results of an experiment in which pigs were fed FB₁
14 in a dose of 2–3 mg kg⁻¹ of feed, and the toxin could be detected in the liver and kidney
15 predominantly in unchanged chemical form even 2 weeks after toxin feeding had been
16 discontinued (Prelusky et al. 1996b).
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27 In vervet monkeys there is evidence of metabolism to partially hydrolysed (one propane
28 tricarboxylic acid residue removed) FB₁, and to a much lesser extent the fully
29 hydrolysed (both propane tricarboxylic acid residues removed) aminopentol backbone,
30 in faeces while in urine 96% of the ¹⁴C label was recovered as FB₁ (Shephard et al.
31 1994b).
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39 It is supposed that FB₁ is metabolised in the intestine, as both the partially and the
40 completely hydrolysed FB₁ can be detected in the faeces and not in the bile (Shephard et
41 al. 1995).
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47 **Conclusions**

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3 Feeding of FB₁ to pigs in a combined toxin dose of 50 mg FB₁, 20 mg FB₂ and 5 mg
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5 FB₃/animal/day for 22 days did not cause clinical signs and feed refusal despite the fact
6
7 that the typical disease entity, i.e. pulmonary oedema of varying severity developed in
8
9 all the animals. The haematological variables were within the physiological range. Due
10
11 to the effect of contaminated feed, the ALT activity of the blood plasma increased
12
13 significantly as compared to the control group.
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17 In partial agreement with the relevant data of the literature, the FB₁ was accumulated in
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19 the liver and kidney, while the highest average amount of FB₂ was found in the liver
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21 and the fat samples. In agreement with the results of our previous studies, the muscle and
22
23 adipose tissue (i.e. the meat) did not contain appreciable amounts of fumonisin B₁, and
24
25 thus it does not pose a major risk to human health.
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29 During the continuous feeding of the mentioned toxin combinations, 13% of the total
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31 quantity of FB₁ taken up during the 5 days was excreted unchanged with faeces and
32
33 urine, and from this quantity 86 % was recovered in the faeces and 14 % in the urine.
34
35 From FB₂ taken up during this period a strongly lower average quantity was detected in
36
37 these excreta. Supposedly, the elimination of unchanged form of FB₂ was less than that
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39 of FB₁.
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43 It must be noted that the actual elimination would presumably be much higher than that
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45 if the metabolised chemical forms were also taken into consideration. Further studies
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47 are needed to clarify the fate of the unaccounted portion of the dose.
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