



Absorption, distribution and elimination of fumonisin B1 metabolites in weaned piglets

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Additives/Contaminants:	Fumonisin, Mycotoxins - fumonisin
Food Types:	Animal products ♦ meat, Animal feed

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4 1 **Absorption, distribution and elimination of fumonisin B₁ metabolites in**
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6 2 **weaned piglets**
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11 4 **Abstract**
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13 The absorption, distribution and elimination of fumonisin B₁ (and B₂) after oral
14 administration of *Fusarium verticillioides* (MRC 826) fungal culture, mixed into the
15 experimental feed for 10 days, was studied in weaned barrows. In order to determine the
16 absorption of FB₁ from the feed marked by chromium oxide, a special T-cannula was
17 implanted into the distal part of pigs' ileum. During the feeding of toxin-containing diet (45
18 mg FB₁ per kg) and until the 10th day after the end of treatment, the total quantity of urine and
19 faeces was collected and their toxin content was analysed. At the end of the trial, samples of
20 lung, liver, kidney, brain, muscle and fat were also collected and their fumonisin content was
21 analysed by LC-MS. The fumonisins appeared to decrease the reduced glutathione content in
22 blood plasma and red blood cell haemolysate, possibly associated with *in vivo* lipid
23 peroxidation. From a dataset of eighty individual data and the concentration and rate of Cr
24 and fumonisins (FB₁, partially hydrolysed FB₁ and aminopentol) in the chymus, it could be
25 established that the accumulative absorption of fumonisin B₁ was 3.9±0.7 %. In the chymus,
26 the FB₁ conversion into aminopentol and partially hydrolysed FB₁ was 1% and 3.9%,
27 respectively. The degree of metabolism in faeces was variable, although the main product was
28 the partially hydrolysed form, with very small amounts of the aminopentol moiety being
29 recovered. In the investigated tissues the FB₁ conversion to aminopentol and partially
30 hydrolysed FB₁ was 30% and 20%, respectively.
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24 **Keywords:** fumonisin, metabolites, biotransformation, absorption, lipid peroxidation
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6 2**Introduction**

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8 3 Fumonisin were first isolated in 1988 from a culture of *Fusarium verticillioides* (earlier
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10 4 *Fusarium moniliforme*) strain MRC 826 grown on maize (Gelderblom et al. 1988, Cawood et
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12 5 al. 1991), and subsequently their structure was also determined (Bezuidenhout et al. 1988).
13
14 6 The fumonisin B analogues, including toxicologically important FB₁, FB₂ and FB₃ are the
15
16 7 most abundant naturally occurring fumonisins (Marasas, 1996). Fumonisin B₁ (FB₁) causes
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18 8 leukoencephalomalacia in horses (Marasas et al. 1988) and pulmonary oedema in swine
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20 9 (Harrison et al. 1990). It is carcinogenic and has been implicated in the pathogenesis of
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22 10 oesophageal cancer in humans (Marasas et al. 1996).
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29 12 The results of surveys indicate that fumonisins contaminate the maize kernels in all corn-
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31 13 growing countries of the world and can cause fumonisin toxicosis (Dutton 1996). The exact
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33 14 mechanism of action of FB₁ has still not been elucidated. Fumonisin are very similar to
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35 15 sphingolipids in molecular structure (Shier 1992); thus, they interfere in the metabolism of the
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37 16 latter and disturb the processes mediated by these molecules (Kim et al. 1991).
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43 18 Food materials of animal origin may become contaminated with FB₁ after the toxin has been
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45 19 absorbed from the digestive tract, entered the bloodstream and reached the peripheral tissues.
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47 20 Therefore, from the food safety point of view it is especially important to know the
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49 21 distribution of this mycotoxin in the organism. However, the toxin concentration detectable in
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51 22 the organs, urine and faeces is influenced by the metabolism of the toxin to a large extent.
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53 23 Only a few data have been so far reported in the literature about the metabolism of FB₁
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55 24 (Marasas et al., 2000).
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1 The FB₁ molecule includes a long chain aminopentol backbone (AP₁) with two ester-linked
2 tricarballic acids (TCA). AP₁ originates from FB₁ by hydrolysis of the tricarballic acid
3 side chains at carbon 14 and 15, which are then replaced by hydroxyl groups. PHFB₁
4 (partially hydrolysed FB₁) is a result of partial hydrolysis (Merrill et al., 1993).

5 Little is known about the possible endogenous hydrolysis of FB₁ by the mammalian
6 metabolism, even if some studies performed on primates (Shepard et al. 1994) and ruminants
7 (Rice and Ross, 1994) revealed that the ester moiety of FB₁ was hydrolyzed in the intestine.

8
9 In our earlier study (Fodor et al., 2006a), from the toxin intake (50 mg per animal per day or
10 2.2 mg per kg body weight per day) of five days, a mean value of 13% was excreted in urine
11 and faeces. Distribution of the excreted toxin in faeces and urine was 87% and 13%,
12 respectively. Our examinations have applied to the unchanged chemical form of FB₁ only. It
13 must be noted that the actual elimination would have presumably been much higher than that
14 if the metabolised chemical forms had also been taken into consideration. Accordingly, it was
15 supposed that the major part of the toxin was excreted in a partly or totally hydrolysed form.

16 In this experiment, absorption, distribution and elimination of fumonisin B₁ and its
17 metabolites were determined in pigs that were fed a ration containing 45 mg FB₁ kg⁻¹ during
18 10 days.

19 **Materials and methods**

20 *Experimental design and animals*

21 Sixteen weaned barrows (Hungarian Large White) from the age of 8 weeks, weighing 12–14
22 kg, were used in the experiment. The piglets were weighed and then divided into two groups:
23 an experimental group (n = 10) and a control group (n = 6). The animals were placed in
24 metabolic cages for the trial. The room temperature (20 °C) was controlled according to the

1 needs of weaned piglets. Feed was given twice a day, in two equal portions, and the amount
2 of feed not consumed by the animals was measured. Drinking water was available *ad libitum*
3 via automatic drinkers. The experimental animals were fed a basal diet of a composition
4 corresponding to their age (187 g kg⁻¹ crude protein, 12.8 MJ kg⁻¹ metabolizable energy, 13.1
5 g kg⁻¹ lysine; ingredients: wheat 30%, barley 20%, corn 15%, soy-bean 10%, concentrate
6 15%). A *Fusarium verticillioides* (MRC 826) fungal culture (1%) and Cr₂O₃ (0.5%) was
7 mixed into the feed of experimental animals, so as to provide a Cr-labelled, fumonisin-
8 contaminated (45 mg/kg FB₁; 8.6 mg/kg FB₂; 4.6 mg/kg FB₃) feed. Fumonisin were
9 produced by a locally developed method and with the application of *Fusarium verticillioides*
10 strain MRC 826 (Fodor et al., 2006b). The homogenized fungal culture contained FB₁, FB₂
11 and FB₃ at a concentration of 5645 mg kg⁻¹, 1083 mg kg⁻¹ and 581 mg kg⁻¹, respectively.

12 After a five-day adaptation period, according to the method of Tossenberger et al. (2000), a
13 special T-cannula was implanted into the distal part of the ileum, before the ileocaecal valve,
14 in order to determine the absorption of FB₁ from the feed marked by Cr₂O₃. At days 1 and 10
15 of the intoxication period, and 10 days after the withdrawal of the contaminated diet animals
16 were weighed and blood samples were taken. From the blood samples lipid peroxidation
17 parameters were measured, as a complementary examination. From the beginning of the
18 experiment to end, total quantity of urine and faeces was collected, in order to determine the
19 toxin content of them. At the end of both of periods, piglets (n=5 treated and 3 control
20 animals) were killed by bleeding after sedation. Gross pathological examinations were
21 performed, organs were weighed and several organs were sampled, in order to determine their
22 toxin content and lipid peroxidation status.

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24 The experiments were carried out according to the regulations of the Hungarian Animal
25 Protection Act. The allowance number for the studies was MÁB-11/2002; KÁ-16/2001.

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6 2 *Determination of mycotoxin content in feeds and fungal cultures*
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9 3 The mycotoxin content of the control and the experimental feed was checked. T-2 mycotoxin
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11 4 was determined with GC-MS (LOD: 0.01 mg kg⁻¹), while zearalenone (LOD: 0.01 mg kg⁻¹),
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13 5 DON (LOD: 0.05 mg kg⁻¹) and ochratoxin A (LOD: 0.1 µg kg⁻¹) by a HPLC system.
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18 7 The determination of FB₁, FB₂ and FB₃ content from the culture and feed samples was
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20 8 confirmed also by a HPLC fluorescence detection method based upon derivatisation with o-
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22 9 phthaldialdehyde (OPA), as described earlier (Fodor et al., 2006a).
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27 11 The diet fed to the control group did not contain detectable amounts of mycotoxin.
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31 32 13 *Lipid peroxidation examinations*

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34 14 For the determination of lipid peroxidation, the samples of blood and organs were stored at –
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36 15 82 °C until analysis. Lipid peroxidation was determined by the quantification of
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38 16 malondialdehyde (MDA) levels with 2-thiobarbituric acid method in blood plasma (Placer et
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40 17 al., 1966), liver, lung, brain, kidney and spleen (Mihara et al., 1980). Among the small
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42 18 molecular weight antioxidants, the amount of reduced glutathione (Sedlak and Lindsay, 1968)
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44 19 was measured in blood plasma, and red blood cell haemolysate and in the above-mentioned
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46 20 organs.
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50 51 52 53 22 *Determination of fumonisin absorption and excretion*

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56 23 For the investigation of absorption of FB₁, weaned barrows implanted with a special T-
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58 24 cannula (PVTC) into the distal part of the ileum were used. The operation was carried out 7
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60 25 days before toxin feeding. In the intoxication period (for 10 days) chymus samples were

1 collected twice a day (2-3 hrs after feed consumption) from every animal. Based on the rate of
2 FB₁ and Cr₂O₃ in the feed, the absorption degree of the fumonisin was quantifiable from the
3 chymus toxin content. In the experimental diet the concentration of Cr and FB₁ was 3.84 g kg⁻¹
4 and 45 mg kg⁻¹, respectively. During the calculation of FB₁ absorption (as calculated from
5 the Cr-FB₁ ratio in the feed) the 85:1 of Cr:FB₁ ratio was taken as a basis. Therefore, the
6 absorption was calculated (%) based on deviation from the rate of 85:1.

7
8 The preparation of the samples was carried out by the method of Christian and Coup (1954),
9 and for the determination of the chymus Cr content atomabsorption method (Atomabsorption
10 Spectrophotometer, AA-6701F Shimadzu) was used. For the atomizing, acetylene-air flame
11 was applied. From day 2, during the calculation it was corrected with the toxin amount
12 excreted back into the intestine by bile (literature data; Dantzer et al., 1999; biliary excretion
13 of FB₁: 1.4% of the dose at 4 h after dosing). It was also taken the recovery rate of Cr (at the
14 end of the ileum; Cr recovery: 98.6%; Köhler, 1993) into consideration.

15
16 In order to monitor the excretion of fumonisins, during the trial the total quantity of urine and
17 faeces was collected. Faeces were collected two times daily (following the morning and
18 afternoon feeding), and weighed with gram precision. Every day the entire quantity of faeces
19 (stored at 4 °C until further processing) was homogenised and 10 g was sampled and frozen
20 (at -18 °C) until laboratory analysis. Urine was collected continuously into a sealed container
21 connected, and its volume was measured following the morning feeding. The urine collected
22 daily was homogenised and 100 ml was stored at -18 °C until the analysis.

23
24 *Analysis of the fumonisin content of organs, faeces, urine and chymus*

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3 1 The amount of toxins detectable in different organs and tissues including the liver, lungs,
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5 2 kidney, brain, spleen, the *m. longissimus dorsi* and *m. psoas major* muscles, and abdominal
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7 3 and subcutaneous fat was determined. Furthermore, the toxin content of faecal, urine and
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9 4 chymus samples was measured.
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15 6 Quantification and identification of intact fumonisin B₁ and fumonisin B₂ and metabolites of
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17 7 FB₁, namely aminopentol and partially hydrolysed fumonisin B₁ was carried out using LC
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19 8 (PerkinElmer, Series 200; USA)-MS (API 3200 LC/MS/MS System; Applied Biosystems,
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21 9 USA). In the absence of analytic standard for FB₂ metabolites, the absorption of FB₂ was not
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23 10 determined. The determination of tissues' and urines toxin content is equal of the described in
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25 11 the method of Fodor et al. (2006a).
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33 13 Non-dried and non-lyophilized faeces (2 g) and chymus (5 g) samples were extracted by a
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35 14 mixture of 0.1 M ethylenediaminetetraacetate (EDTA) and methanol (8ml; 3/1 v/v) for 60
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37 15 min. The samples were filtrated through a fiberglass filter (Whatman GF A, Dassel, Germany)
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39 16 after centrifugation (2000 g; 3 min). An aliquot of the filtrate was analysed.
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46 18 Similarly to our earlier experiment (Fodor et al., 2006a), pure FB₁ and FB₂ (Sigma,
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48 19 Schnellendorf, Germany) were used for the determination. PHFB₁ (partially hydrolysed FB₁)
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50 20 standard was prepared by Stephen M. Poling (U.S.A.), according to the method of Poling and
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52 21 Plattner (1999). This standard was an equilibrium mixture of the two partially hydrolyzed FB₁
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54 22 forms. (PHFB_{1a} - TCA group at the C-15 hydroxy group (hydrolysis at C-14); PHFB_{1b} - TCA
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56 23 group at the C-14 (i.e. hydrolysis at C15)). AP₁ standard for the analysis was made by the
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1 method of Pagliuca et al. (2005). Every standard was diluted with a mixture of ACN/H₂O
 2 (1:1). Table 1 shows the validation data for the FB₁ and its metabolites, and FB₂.

3
 4 Taking the low feed concentrations of FB₃ into account, as compared to the other FB forms,
 5 and its presence supposedly under the LOD value, these parameters were not aimed to
 6 determine.

7 [Insert Table 1 about here]

9 *Determination of FB₁ conversion into metabolites*

10 Based on the molecular weights of the fumonisin B₁ compounds (fumonisin B₁ (α): 721
 11 g/mol; aminopentol (β): 405 g/mol; aminopolyols (γ): 563 g/mol) FB₁ conversion into its
 12 metabolites was calculated, as described below:

13
 14 the fumonisin B₁ – aminopentol conversion:

$$15 \lambda\alpha \rightarrow \beta = \frac{m\beta / M\beta}{m\alpha / M\alpha} = \frac{m\beta}{m\alpha} \times \frac{M\alpha}{M\beta}$$

16 the fumonisin B₁ – partially hydrolyzed FB₁ conversion:

$$17 \lambda\alpha \rightarrow \gamma = \frac{m\gamma / M\gamma}{m\alpha / M\alpha} = \frac{m\gamma}{m\alpha} \times \frac{M\alpha}{M\gamma}$$

18 where *m* indicates the mass of compounds in 1 g and *M* means their relative molecular
 19 weights.

21 *Statistical analysis*

22 The entire measurement dataset was analysed statistically. Correlation analysis ($P \leq 0.05$) was
 23 performed to determine the correlation between the concentration of FB₁ and PHFB₁ or AP₁
 24 in the faeces samples. Data processing and the mathematical-statistical calculations were

1 performed using the Correlate and Descriptive Statistics modules of the SPSS 10.0
2 programme package and the spreadsheet and figure editor programmes of EXCEL 7.0.
3 Statistical evaluation of the results derived from blood sampling was carried out by analysis
4 of variance (ANOVA) and least significant difference (LSD) “post hoc” tests.

5 **Results and Discussion**

6 *Clinical signs and findings of the pathomorphological examinations*

7 Pigs consumed 36.6 ± 6.5 mg FB₁ per day did not show clinical signs of disease and their feed
8 consumption was balanced throughout the experiment. At the first examination (after the 10-
9 day long toxin exposure) pulmonary oedema, the typical disease entity caused by fumonisin-
10 contaminated feed, developed in all of the piglets (n=5) as a result of the toxin dose applied.
11 In the pleural cavity of animals a small volume (2–7 ml) of slightly yellowish fluid with
12 clotting characteristics could be found. Of the other organs examined, beside the pathological
13 change of the liver, the heart, and the kidneys, the spleen showed hyperplasia. 10 days after
14 the end of the toxin feeding, in case of 4 animals 8-9 ml fluid was distinctly visible in the
15 pericardium. The pulmonary oedema was estimated as minor degree.

17 *Antioxidant results*

18 Based on results of the lipid peroxidation measures, it could be concluded that the glutathione
19 content of blood plasma and haemolysed red blood cell was decreased significantly (Figure 1)
20 but other changes indicative of lipid peroxidation were found neither in the period of toxin
21 exposure nor after that time. In the concentration of reduced glutathione a significant
22 (P=0.0007) decrease was experienced between the first and second sampling in case of treated
23 group (Figure 1), while in the control group this value remained at an identical level with the

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3 1 first blood sampling. Similar decrease ($P= 0.043$) was found in GSH levels/concentration
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6 2 between these two time points in the RBC haemolysates.

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8 3 [Insert Figure 1 about here]

9
10 4 Summarized, fumonisin in the applied dose did not cause the production of peroxy radicals in
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12 5 significant measure in these tissues but in blood (plasma and haemolysed RBC) it resulted
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14 6 reduction in the second line of the antioxidant system, namely in the reduced glutathione. This
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16 7 hypothesis is supported by the findings that fumonisin B₁ induces oxidative stress in human,
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18 8 rat and mouse neural cell cultures. Decreased GSH levels, indicative of lipid peroxidation and
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20 9 necrotic cell death were observed in all cell lines after incubation with FB₁ (Stockmann et al.,
21
22 10 2004).

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27 11 Our results provide the first evidence that the fumonisins *in vivo* appear to decrease the GSH
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29 12 content in blood and haemolysed RBC, associated with lipid peroxidation.

30 31 32 33 34 14 *Absorption of fumonisin B₁*

35
36 15 Table 2 shows the results of the calculated daily absorption (per animal) of FB₁ in the 10-day
37
38 16 period. By eighty individual data (taking the molecular weight of FB₁, PHFB₁ and AP₁ into
39
40 17 account) it was established that the mean accumulative absorption rate of all animals tested
41
42 18 for intact fumonisin B₁ and its metabolites formed in the small intestine (till the end of the
43
44 19 ileum) is 4%. There was no significant correlation between the daily fumonisin intake and the
45
46 20 absorption of the toxin. Until now, about the exact absorption of FB₁ only computed data
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48 21 were available; this is the first result from an absorption measurement.

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53 22 [Insert Table 2 about here]

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55 23 Following gavage dosing to vervet monkeys, only small amounts of FB₁ were absorbed, and
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57 24 after 24 hr major organs retained little of the radiolabel, with the liver retaining the most
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59 25 (Shephard et al., 1995). The p.o bioavailability of FB₁ was 3.5% after single doses of 10 mg

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3 1 FB_1 kg^{-1} to rats, estimated by serial blood, liver and kidney tissue samples (Martinez-
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6 2 Larranaga et al., 1999). Based on plasma and excretion data of pigs, systematic bioavailability
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8 3 following intragastrical dosing was estimated to be higher, as compared to the above
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10 4 mentioned, approximately 3-6 % (Prelusky et al., 1994).

11
12 5 In the chymus, the FB_1 conversion into aminopentol and partially hydrolysed FB_1 was 1% and
13
14 6 3.9%, respectively (data not shown). Accordingly, the biotransformation (metabolism) of
15
16 7 fumonisin B_1 was proven to happen already in the small intestine; the absorption of
17
18 8 metabolites (mainly of the aminopentol) might also occur.

19
20 9 No data have so far been reported on the FB_1 metabolite of small intestinal content. In the
21
22 10 experiment of Shephard et al (1995), at the time of sacrifice, the stomachs and small intestines
23
24 11 of the monkeys were empty, so the scheme of chymus sampling was not successful. All bile
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26 12 samples failed to show the presence of any hydrolysis products in the cited study. Based on
27
28 13 this fact, Shephard et al. supposed already in 1995 that the hydrolysis process occurs only in
29
30 14 the gut and not in the liver.

31 32 33 34 35 36 37 38 16 *Fumonisin content of the organs examined*

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40
41 17 With respect to the toxin content of organs, much lower FB_1 concentrations (maximum level
42
43 18 in the liver) were measured, as compared to those in our earlier experiment (Fodor et al.,
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45 19 2006a). This was probably due to the difference between the daily feed intake (in the earlier
46
47 20 trial 2 and at present 2.2 mg kg^{-1} bw.) and the length (for 22 and 10 days, respectively) of the
48
49 21 toxin load in the two trials.

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52 22 [Insert Table 3 about here]

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55 23 In the investigated tissues 50% of the recovered FB_1 was intact (Table 3). FB_1 conversion into
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57 24 aminopentol and partially hydrolysed FB_1 was 30% and 20%, respectively. In most of the
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3 1 organs detectable amounts of FB₁ (50%) and its metabolite, aminopentol (50%) were
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5 2 measured even 10 days after the dosage of the non-contaminated feed.
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10 4 *Excretion of fumonisins*
11

12 5 During the 10-day toxin-feeding period, the daily toxin intake of the animals was 36.6 ±6.5
13 6 mg (2.2 mg kg⁻¹ bw.) in average. In the first five days after beginning of the toxin feeding, the
14 7 FB₁ concentration of the faecal content increased continuously, and then from the 5th day to
15 8 the 10th day it was about 55 µg g⁻¹ (Figure 2). There was no change in the aminopentol
16 9 concentration after the 3rd day, it was measured in standard but low (6 µg g⁻¹) concentration.
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22 10 [Insert Figure 2 about here]
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27 11 Taking the molecular weight of the compounds into account, it was established that during the
28 12 continuous toxin exposure (in 10 days) 59% of the total fumonisin B₁ compounds recovered
29 13 in the faeces were determined as partially (47%) or totally (12%) hydrolysed metabolites.
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33 14 Previous toxicokinetic studies on vervet monkeys, based on iv and oral dosing of FB₁,
34 15 similarly demonstrated the rapid elimination of FB₁ in the faeces (Shephard et al., 1995). The
35 16 extent of hydrolysis varied considerably, although the main product was the partially
36 17 hydrolysed form (approximately 1/3 of total fumonisins), with very small recovered amounts
37 18 of the fully hydrolysed (aminopentol) moiety.
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49 20 Investigating the period between the 5th and 10th day, and having regard to all fumonisins,
50 21 72% (27.3 mg) of FB₁ taken up was recovered daily in the faeces. Between the days 5 and 10,
51 22 a slower but constant increase was noted in the daily-excreted amount, suggesting a balance
52 23 between the FB₁ intake and its elimination.
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1 During the time of toxin exposure a relatively close correlation ($r = -0.4$, $P < 0.05$) was found
2 between the concentration of FB_1 and $PHFB_1$ in the faeces samples, while there was no
3 statistically significant correlation between the concentration of FB_1 and AP_1 .

4
5 After changing the experimental feed for the non-contaminated, a significant decrease was
6 observed in the concentration of FB_1 and its metabolites already on the 3rd day. Three days
7 after the end of toxin feeding, $PHFB_1$ was the dominant compound (75%) in the faeces. Less
8 than 1 mg fumonisins were excreted daily after the 15th day via faeces. Detectable amounts of
9 FB_1 and its metabolites were measured in the faeces even 10 days after the dosage of the non-
10 contaminated feed.

11
12 23% of the FB_2 consumed during the trial was eliminated by the faeces. This value is much
13 lower than in the results of Shephard and Snijman. (1999), where the recoveries of
14 unmetabolized FB_2 from faeces of 2 vervet monkeys were 8% and 56% (mean: 32%) over a
15 7-day period. This paper also addresses the occurrence of partially hydrolysed FB_2 , which
16 accounts for some of the remainder of the dose.

17
18 1.5% of FB_1 quantity taken up was excreted with the urine during the entire trial, about 65%
19 in original, 16% in totally hydrolysed and 24% in partially hydrolysed form, while 0.6% of
20 the FB_2 consumed during the trial was eliminated via urine, as intact molecule.

21
22 Summarized, from the 360 mg FB_1 taken up in 10 days, 247.5 mg (69%) FB_1 (plus
23 metabolites) was excreted by the faeces and urine during the toxin load and the 10-day long
24 elimination period. In the first 10 days 222 mg (90%), while after this time 25.5 mg (10%)
25 fumonisins were recovered.

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6 2 **Conclusion**
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8 3 In our first *in vivo* experiment (Fodor et al., 2006a) on the toxin elimination, by the
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10 4 determination of the intact FB₁ moieties, a strongly negative balance was found in the total
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12 5 toxin amount, when taking the moieties excreted in the urine and faeces into account. This
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14 6 was, at least in part, the basis of the present experiment, where the most important and
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16 7 frequent metabolites were also determined.
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20 9 From the trials on animals, we could draw a conclusion that the intestinal microbiota of pigs
21
22 10 is able to transform the intact FB₁ to a similarly toxic substance (partially hydrolysed FB₁) or
23
24 11 a more toxic metabolite (aminopentol). As a general principle, from the two metabolites,
25
26 12 partially hydrolysed FB₁ has priority during the metabolic process. The conversion of FB₁ to
27
28 13 AP₁ is notable even despite of its little amount, because this new compound means a new risk
29
30 14 from the viewpoint of animal- and human health as well, taking into account that aminopentol
31
32 15 is hydrophobic molecule (with a more effective absorption) and that its further derivative (N-
33
34 16 palmitoyl-aminopentol) appears to be tenfold toxic than the original FB₁ (Humpf et al., 1998).
35
36 17 On the basis of the calculated accumulative absorption rate (maximally 4%) it is not clear,
37
38 18 which metabolites shaped exactly this value. From the point of view of human exposure,
39
40 19 special attention should be paid to AP₁ appeared in edible tissues even 10 days after the
41
42 20 dosage of the non-contaminated feed because there is a lack of information about the effect of
43
44 21 this metabolite consumed in low concentration but for a long term.
45
46 22

47
48 23 AP₁ was less potent than FB₁ as an inhibitor of ceramide synthase *in vitro* (Merrill et al.,
49
50 24 1993). A feeding study in which rats were fed corn containing 50 ppm of FB₁ or 10 ppm AP₁
51
52 25 resulted in roughly equal toxicity signs in both groups, indicating that AP₁ could be more

1 toxic than FB₁ itself (Hendrich et al. 1993). AP₁ shows also cancer-promoting activity in liver
2 (Hendrich et al. 1993). Removing the tricarballylic acids, AP₁ becomes not only an inhibitor
3 but also a substrate for acylation by ceramide synthase. In the presence of palmitoyl-CoA AP₁
4 is also acylated to form N-palmitoyl-AP₁ (PAP₁), which is a more potent inhibitor of
5 ceramide synthase (Humpf et al. 1998). Thus, if FB₁ is efficiently metabolized to
6 aminopentol, the above mechanism may lead to metabolic activation.

7
8 Further investigations are reasoned to clarify the dose-dependent metabolism and absorption
9 of fumonisin B₁.

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18 **References**

19 BEZUIDENHOUT, S. C., GELDERBLOM, W. C. A., GORSTALLMAN, C. P., HORAK, R. M.,
20 MARASAS, WFO, SPITELLER, G. and VLEGGAAR, R., 1988, Structure elucidation of the
21 fumonisin mycotoxins from *Fusarium moniliforme*. *Journal of the Chemical Society,*
22 *Chemical Communications*, **11**, 743-745.

- 1
2
3 1 CAWOOD, M.E., GELDERBLOM, W.C.A., VLEGGAR, R., BEHREND, Y., THIEL, P.G. and
4
5 2 MARASAW, W.F.O., 1991, Isolation of the fumonisin mycotoxins: a quantitative
6
7 3 approach. *Journal of Agricultural and Food Chemistry*, **39**, 1958–1962.
8
9
10 4 CHRISTIAN, K. R., and M. R. COUP., 1954, Measurement of feed intake by grazing cattle and
11
12 5 sheep. VI. The determination of chromic oxide in faeces. *N.Z. J. Sci. Technol.* **36**:328.
13
14
15 6 DANTZER WR, HOPPER J, MULLIN K, HENDRICH S, and MURPHY PA., 1999, Excretion of
16
17 7 (14)C-fumonizin B(1), (14)C-hydrolyzed fumonizin B(1), and (14)C-fumonizin B(1)-
18
19 8 fructose in rats. *J Agric Food Chem.* **47**(10):4291-6.
20
21
22 9 DUTTON, M.F., 1996, Fumonisin, mycotoxins of increasing importance: their nature and their
23
24 10 effects. *Pharmacology and Therapeutics*, **70**, 137–161.
25
26
27 11 FODOR J., K. MEYER, M. RIEDLBERGER, J. BAUER, HORN P., KOVÁCS F., and KOVÁCS M.,
28
29 12 2006a, Distribution and elimination of fumonisin analogues in weaned piglets after
30
31 13 oral administration of *Fusarium verticillioides* fungal culture. *Food Additives and*
32
33 14 *Contaminants*, **23**(5):492-501.
34
35
36 15 FODOR, J., KAMETLER, L., and KOVÁCS, M., 2006b, Practical aspects of fumonisin production
37
38 16 under laboratory conditions. *Mycotoxin Research*. 2006. **22**:211-216.
39
40
41 17 GELDERBLOM, W.C.A., JASKIEWICZ, K., MARASAS, W.F.O., THIEL, P.G., HORAK, R.M.,
42
43 18 VLEGGAR, R. and KRIEK, N.P.J., 1988, Fumonisin – novel mycotoxins with cancer-
44
45 19 promoting activity produced by *Fusarium moniliforme*. *Applied and Environmental*
46
47 20 *Microbiology*, **54**, 1806–1811.
48
49
50 21 HARRISON, L.R., COLVIN, B.M., GREENE, J.T., NEWMAN, L.E. and COLE, J.R., 1990,
51
52 22 Pulmonary edema and hydrothorax in swine produced by fumonisin-B1, a toxic
53
54 23 metabolite of *Fusarium moniliforme*. *Journal of Veterinary Diagnostic Investigation*,
55
56 24 **2**, 217-221.
57
58
59
60

- 1
2
3 1 HENDRICH, S., MILLER, K. A., WILSON, T. M., and MURPHY, P. A., 1993, Toxicity of *Fusarium*
4
5 2 proliferatum-fermented nixtamalized corn-based diets fed to rats: Effect of nutritional
6
7 3 status. *J. Agric. Food Chem.* **41**: 1649–1654.
8
9
10 4 HUMPF, H.-U., SCHMELZ, E.-M., MEREDITH, F.I., VESPER, H., VALES, T.R., MENALDINO, D.S.,
11
12 5 LIOTTA, D.C., and MERRILL, A.H. JR., 1998, Acylation of naturally occurring and
13
14 6 synthetic 1-deoxysphinganine by ceramide synthase: formation of N-palmitoyl-
15
16 7 aminopentol (PAP1) produces a toxic metabolite of hydrolyzed fumonizin (AP1), and
17
18 8 a new category of ceramide synthase inhibitor. *J. Biol. Chem.*, **273**: 19060-19064 .
19
20
21 9 KIM, M.Y., LINARDIC, C., OBEID, L. and HANNUN, Y., 1991, Identification of sphingomyelin
22
23 10 turnover as an effector mechanism for the action of tumor-necrosis-factor-alpha and
24
25 11 gamma-interferon – specific role in cell-differentiation. *Journal of Biological*
26
27 12 *Chemistry*, **266**, 484-489.
28
29
30 13 KÖHLER, T. 1993. Evaluation of techniques to collect ileal digesta in pigs. Ph.D.thesis of
31
32 14 Anim. Nutr. Dept. Wageningen Agric. Univ.
33
34
35 15 MARASAS, W.F.O., KELLERMAN, T.S., GELDERBLOM, W.C.A., COETZER, J.A.W., THIEL, P.G.
36
37 16 and VAN DER LUGT, J.J., 1988, Leukoencephalomalacia in a horse induced by
38
39 17 fumonisin B1 isolated from *Fusarium moniliforme*. *Onderstepoort. Journal of*
40
41 18 *Veterinary Research*, **55**, 197–203.
42
43
44 19 MARASAS, W. F. O., 1996, Fumonisin: history, worldwide occurrence and impact, p. 1-17. *In*
45
46 20 L. S. Jackson, J. W. DeVries, and L. B. Bullerman (ed.), *Fumonisin in food*. Plenum
47
48 21 Press, New York, N.Y.
49
50
51 22 MARASAS, W. F. O., MILLER, J. D., RILEY, R. T., VISCONTI, A. (eds). 2000. Fumonisin B₁,
52
53 23 International Programme on Chemical Safety (IPCS, UNEP, ILO, and WHO).
54
55 24 Environmental Health Criteria 219 (Geneva: WHO)
56
57
58
59
60

- 1
2
3 1 MARASAS, W. F. O., MILLER, J. D., RILEY, R. T., and VISCONTI, A. (EDS), 2000, Fumonisin B1,
4
5 2 International Programme on Chemical Safety (IPCS, UNEP, ILO, and WHO).
6
7 3 Environmental Health Criteria 219 (Geneva: WHO)
8
9
10 4 MARTINEZ-LARRANAGA, M.R., ANADON, A., DIAZ, M.J., FERNANDEZ-CRUZ, M.L., MARTINEZ,
11
12 5 M.A., FREJO, M.T., MARTINEZ, M., FERNANDEZ, R., ANTON, R.M., MORALES, M.E.
13
14 6 TAFUR, M. 1999. Toxicokinetic and oral bioavailability of fumonisin B1. *Vet. Hum.*
15
16 7 *Toxicol.* 41:357–362.
17
18
19 8 MERRILL A. H. JR., WANG E., GILCHRIST D. G., and RILEY R. T., 1993, Fumonisin and other
20
21 9 inhibitors of de novo sphingolipid biosynthesis. *Adv. Lipid Res.* 26:215-234
22
23
24 10 MIHARA, M., UCHIYAMA, M. AND FUKUZAWA, K., 1980, Thiobarbituric acid value of fresh
25
26 11 homogenate of rat as parameter of lipid peroxidation in ageing, CCl₄ intoxication and
27
28 12 vitamin E deficiency. *Biochemical Medicine* 23 (5), 302–311.
29
30
31 13 PAGLIUCA G, ZIRONI E, CECCOLINI A, MATERA R, SERRAZANETTI GP, and PIVA A., 2005,
32
33 14 Simple method for the simultaneous isolation and determination of fumonisin B1 and
34
35 15 its metabolite aminopentol-1 in swine liver by liquid chromatography--fluorescence
36
37 16 detection. *J Chromatogr B Analyt Technol Biomed Life Sci.* 5;819(1):97-103.
38
39
40 17 PLACER, Z.A., CUSHMAN, L.L., JOHNSON, B.C., 1966, Estimation of product of lipid
41
42 18 peroxidation (malonyl dialdehyde) in biochemical systems. *Analytical Biochemistry*
43
44 19 16 (3), 359–364.
45
46
47 20 POLING, S.M. and PLATTNER, R.D., 1999, Rapid purification of fumonisins and their
48
49 21 hydrolysis products with solid-phase extraction columns. *J. Agric. Food Chem.*
50
51 22 47:2344-2349.
52
53
54 23 PRELUSKY, D.B., TRENHOLM, H.L. and SAVARD, M.E., 1994, Pharmacokinetic fate of ¹⁴C-
55
56 24 labelled fumonisin-B1 in swine. *Natural Toxins*, 2, 73–80.
57
58
59
60

- 1
2
3 1 RICE LG, and ROSS PF., 1994, Methods for detection and quantitation of fumonizins in corn,
4
5
6 2 cereal products and animal excreta. *J Food Prot*, **57**: 536-540.
7
8 3 SEDLAK, I., LINDSAY, R.H., 1968, Estimation of total, protein-bound and non-protein sulfhy-
9
10 4 ryl groups in tissues with Ellmann's reagent. *Analytical Biochemistry* 25 (2), 192–205.
11
12 5 SHEPHARD GS, THIEL PG, SYDENHAM EW, VLEGGAR R, and ALBERTS JF., 1994,
13
14 6 Determination of the mycotoxin fumonizin B1 and identification of its partially
15
16 7 hydrolysed metabolites in the faeces of non-human primates. *Food Chem Toxicol*, **32**:
17
18 8 23-29.
19
20 9 SHEPHARD GS, THIEL PG, SYDENHAM EW, and SAVARD ME., 1995, Fate of a single dose of
21
22 10 [14C]-labelled fumonizin B1 in vervet monkeys. *Nat Toxins*, **3**: 145-150.
23
24 11 SHEPHARD, G.S., SNIJMAN, P.W. 1999. Elimination and excretion of a single dose of the
25
26 12 mycotoxin fumonizin B₂ in a non-human primate. *Food Chem. Toxicol.* 37:111–116.
27
28 13 SHIER, W.T., 1992, Sphingosine analogues: an emerging new classof toxins that includes the
29
30 14 fumonisins. *Journal of Toxicology - Toxin Reviews*, **11**, 241–257.
31
32 15 SPSS for Windows, ver. 10.0., 1999, SPSS Inc., Chicago, IL, USA
33
34 16 STOCKMANN-J. H., MIKKOLA J, NAARALA J, LOIKKANEN J, ELOVAARA E, SAVOLAINEN K.,
35
36 17 2004, Fumonisin B1-induced toxicity and oxidative damage in U-118MG glioblastoma
37
38 18 cells. *Toxicology*. **202**(3):173-83.
39
40 19 TOSSENBERGER J., FÉBEL H., BABINSZKY L., GUNDEL J., HALAS V., and BÓDISNÉ GARBACZ Z.
41
42 20 2000, Ileal digestibility of amino acids in pigs. 1st paper: Determination of ileal
43
44 21 digestibility with different methods. (Review) (in Hungarian, with English abstract).
45
46 22 *Állattenyésztés és takarmányozás*. **49**.375-384.
47
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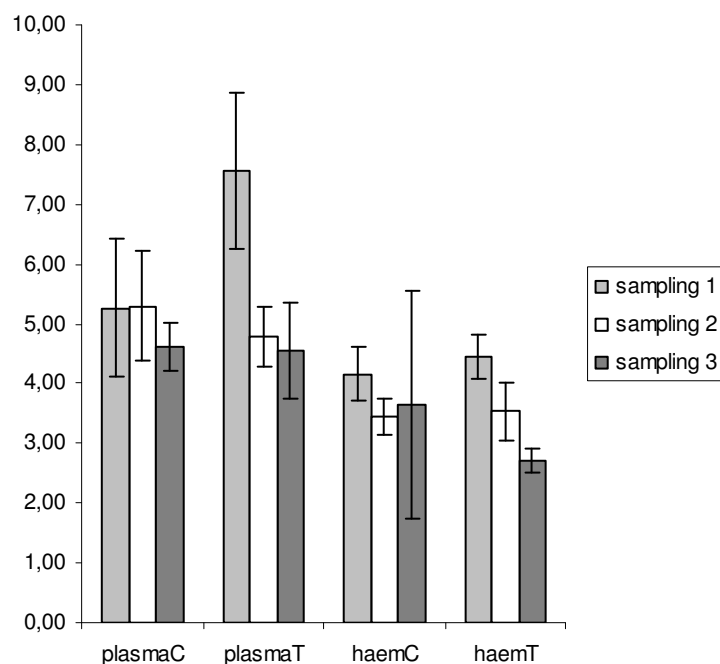


Figure 1. The amount of reduced glutathione (GSH) in blood plasma (plasma) and red blood cell haemolysate (haem) samples (C=control group; T=group fed contaminated feed)

Sampling 1: at the beginning of the intoxication period (day 1); Sampling 2: at the end of the intoxication period (day 10); Sampling 3: 10 days after withdrawal the contaminated feed (day 20)

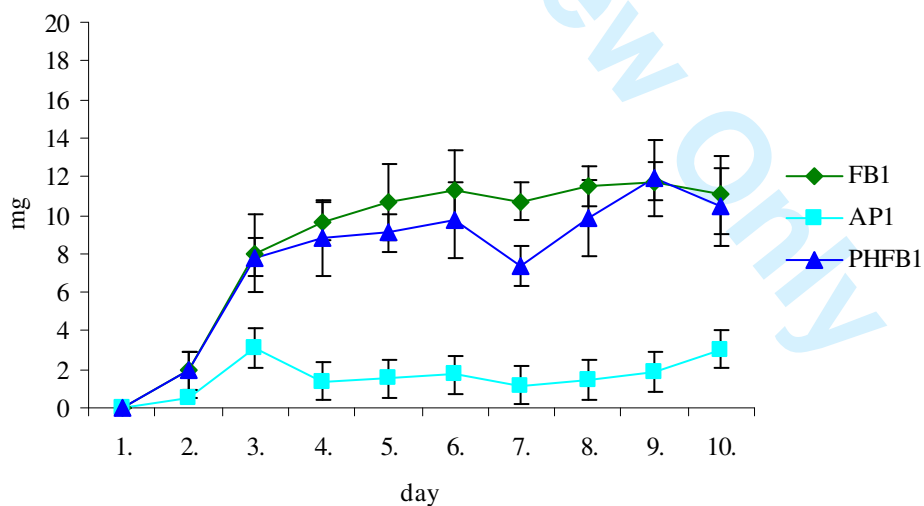


Figure 2. Amount of fumonisins (mg) recovered in faeces during the 10-day long exposure (mean ± S.E.; n=10)

Table 1. Validation data of the analysis

Sample		Recovery \pm SD ^a (%) (n=5)	LOQ ^b ($\mu\text{g kg}^{-1}$) (n=5)	LOD ^c ($\mu\text{g kg}^{-1}$) (n=5)
Faeces, chymus	FB ₁	140.0 \pm 14.7	100	38.6
	FB ₂	107.2 \pm 11.7	1000	155.1
	AP ₁	123.9 \pm 19.2	100	56.0
	PHFB ₁	106.3 \pm 16.2	100	35.6
Urine	FB ₁	98.2 \pm 11.0	10	1.2
	FB ₂	101.3 \pm 9.3	10	3.6
	AP ₁	104.7 \pm 14.0	10	1.9
	PHFB ₁	101.7 \pm 11.8	10	1.2
Lung	FB ₁	92.1 \pm 4.2	10	1.4
	FB ₂	84.1 \pm 12.5	10	2.2
	AP ₁	98.1 \pm 9.1	10	1.3
	PHFB ₁	67.9 \pm 13.3	10	0.9
Liver	FB ₁	90.3 \pm 3.9	10	1.2
	FB ₂	44.1 \pm 2.5	10	2.1
	AP ₁	93.6 \pm 19.6	10	1.4
	PHFB ₁	47.9 \pm 6.0	10	0.8
Kidney	FB ₁	60.3 \pm 13.3	1	0.9
	FB ₂	54.7 \pm 1.4	10	1.4
	AP ₁	115.2 \pm 8.2	1	0.8
	PHFB ₁	47.2 \pm 5.7	1	0.8
Spleen	FB ₁	90.2 \pm 4.7	10	1.0
	FB ₂	72.2 \pm 3.7	10	1.3
	AP ₁	86.7 \pm 8.0	1	0.7
	PHFB ₁	65.7 \pm 5.0	1	0.5
Brain	FB ₁	92.6 \pm 12.6	1	0.6
	FB ₂	91.08 \pm 6.8	10	2.3
	AP ₁	97.1 \pm 15.9	1	0.8
	PHFB ₁	69.9 \pm 10.2	1	0.4
Muscle (m.l.d., m.p.m.)	FB ₁	87.5 \pm 11.0	10	0.6
	FB ₂	60.4 \pm 7.2	10	1.2
	AP ₁	91.3 \pm 8.6	10	0.6
	PHFB ₁	84.3 \pm 15.0	10	0.4
Fat (subcutaneous, abdominal)	FB ₁	84.4 \pm 4.2	1	0.7
	FB ₂	35.1 \pm 2.7	100	3.9
	AP ₁	109.0 \pm 20.1	1	0.6
	PHFB ₁	89.2 \pm 19.9	1	0.6

^a standard deviation^b limit of quantification^c limit of detection

Table 2. Accumulative absorption of the fumonisin B₁ compounds

Animals	Number of chymus samples (n)	Absorption rate* (%)	
		Mean	S.E. ^a
T1	9	8.2	1.7
T2	10	2.6	0.6
T3	6	3.4	0.8
T4	8	0.8	0.2
T5	7	1.9	0.6
T6	8	6.1	1.5
T7	8	2.0	0.6
T8	8	5.7	1.1
T9	6	4.0	0.4
T10	10	5.1	0.9
mean of T1-T10	80	3.9	0.7

^a Standard error

* correcting with the molecular masses

Table 3. The concentration of the different FB₁ derivatives in the organs and the conversion of the FB₁ molecule into metabolites

Organs/Tissues		Fumonisin's concentration (mean \pm SE; $\mu\text{g kg}^{-1}$)* and the FB ₁ conversion (%) into the separate metabolite					
		Sampling 1 (n=5)			Sampling 2 (n=5)		
		mean	\pm SE	%	mean	\pm SE	%
Lung	FB ₁	4.59	1.9		0.93	0.1	
	AP ₁	0.56	1.1	17.0	0.31	0.28 ¹	37.5
	PHFB ₁	0.25	0.2 ³	5.4	ND ⁵	-	
	FB ₂	0.53	0.4 ⁴		ND ⁵	-	
Liver	FB ₁	17.4	1.7		8.25	0.5	
	AP ₁	0.38	0.7 ³	3.1	0.16	0.2 ³	3.3
	PHFB ₁	2.45	1.0 ³	14.9	ND ⁵	-	
	FB ₂	0.86	0.2 ²		ND ⁵	-	
Kidney	FB ₁	9.95	0.3		3.62	0.1	
	AP ₁	7.53	3.2	53.1	2.1	0.9	51.0
	PHFB ₁	1.5	0.04	7.6	ND ⁵	-	
	FB ₂	0.53	0.24 ²		0.34	0.2 ²	
Brain	FB ₁	0.2	0.2 ²		ND ⁵	-	
	AP ₁	0.57	0.4 ²	83.6	0.25	0.1 ¹	
	PHFB ₁	ND ⁵	-		ND ⁵	-	
	FB ₂	ND ⁵	-		ND ⁵	-	
Spleen	FB ₁	4.2	0.5		0.41	0.1 ²	
	AP ₁	1	0.3	23.3	0.18	0.04 ³	41.2
	PHFB ₁	0.55	0.05	9.2	0.04	0.0 ²	6.6
	FB ₂	0.27	0.1		ND ⁵	-	
M. longissimus dorsi	FB ₁	11.2	1.2		0.95	0.2 ²	
	AP ₁	0.72	0.3 ¹	5.4	0.03	0.02 ²	5.3
	PHFB ₁	8.8	1.8	47.7	ND ⁵	-	
	FB ₂	7.9	1.3		0.23	0.1 ³	
M. psoas Major	FB ₁	4.75	1.5		1.41	0.1	
	AP ₁	0.35	0.1	7.9	2.59	1.5	76.7
	PHFB ₁	1.92	0.3 ²	31.0	ND ⁵	-	
	FB ₂	4.06	1.4 ¹		0.28	0.1 ⁴	
Abdominal fat	FB ₁	1.2	0.2		0.9	0.13	
	AP ₁	5.6	3.2	76.1	3.6	0.7	80.6
	PHFB ₁	1.5	0.8	14.7	0.5	0.1	8
	FB ₂	ND ⁵	-		ND ⁵	-	
Subcutaneous fat	FB ₁	2.58	0.7		0.3	0.1 ²	
	AP ₁	0.47	0.3 ³	17.2	0.1	0.1 ²	37.5
	PHFB ₁	1.12	0.8 ³	29.7	ND ⁵	-	
	FB ₂	0.3	0.3 ²		ND ⁵	-	

* superscripts indicate the number of samples in which toxin was not detected

ND not detected

SE standard error

sampling 1. immediately after the 10-day long toxin exposure

sampling 2. 10 days after withdrawal the contaminated feed

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