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

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Abstract

The absorption, distribution and elimination of fumonisin B_1 (and B_2) after oral administration of *Fusarium verticillioides* (MRC 826) fungal culture, mixed into the experimental feed for 10 days, was studied in weaned barrows. In order to determine the absorption of FB_1 from the feed marked by chromium oxide, a special T-cannula was implanted into the distal part of pigs' ileum. During the feeding of toxin-containing diet (45 mg FB_1 per kg) and until the 10^{th} day after the end of treatment, the total quantity of urine and faeces was collected and their toxin content was analysed. At the end of the trial, samples of lung, liver, kidney, brain, muscle and fat were also collected and their fumonisin content was analysed by LC-MS. The fumonisins appeared to decrease the reduced glutathione content in blood plasma and red blood cell haemolysate, possibly associated with *in vivo* lipid peroxidation. From a dataset of eighty individual data and the concentration and rate of Cr and fumonisins (FB_1, partially hydrolysed FB_1 and aminopentol) in the chymus, it could be established that the accumulative absorption of fumonisin B_1 was 3.9±0.7 %. In the chymus, the FB_1 conversion into aminopentol and partially hydrolysed FB_1 was 1% and 3.9%, respectively. The degree of metabolism in faeces was variable, although the main product was the partially hydrolysed form, with very small amounts of the aminopentol moiety being recovered. In the investigated tissues the FB_1 conversion to aminopentol and partially hydrolysed FB_1 was 30% and 20%, respectively.

Keywords: fumonisin, metabolites, biotransformation, absorption, lipid peroxidation

Introduction

Fumonisins were first isolated 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). The fumonisin B analogues, including toxicologically important FB_1, FB_2 and FB_3 are the most abundant naturally occurring fumonisins (Marasas, 1996). Fumonisin B_1 (FB_1) 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. 1996).

The results of surveys indicate that fumonisins contaminate the maize kernels in all corn-growing countries of the world and can cause fumonisin toxicosis (Dutton 1996). The exact mechanism of action of FB_1 has still not been elucidated. Fumonisins 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).

Food materials of animal origin may become contaminated with FB_1 after the toxin has been absorbed from the digestive tract, entered the bloodstream and reached the peripheral tissues. Therefore, from the food safety point of view it is especially important to know the distribution of this mycotoxin in the organism. However, the toxin concentration detectable in the organs, urine and faeces is influenced by the metabolism of the toxin to a large extent. Only a few data have been so far reported in the literature about the metabolism of FB_1 (Marasas et al., 2000).

The FB_1 molecule includes a long chain aminopentol backbone (AP_1) with two ester-linked tricarballylic acids (TCA). AP_1 originates from FB_1 by hydrolysis of the tricarballylic acid side chains at carbon 14 and 15, which are then replaced by hydroxyl groups. $PHFB_1$ (partially hydrolysed FB_1) is a result of partial hydrolysis (Merrill et al., 1993).

Little is known about the possible endogenous hydrolysis of FB_1 by the mammalian metabolism, even if some studies performed on primates (Shepard et al. 1994) and ruminants (Rice and Ross, 1994) revealed that the ester moiety of FB_1 was hydrolyzed in the intestine.

In our earlier study (Fodor et al., 2006a), from the toxin intake (50 mg per animal per day or 2.2 mg per kg body weight per day) of five days, a mean value of 13% was excreted in urine and faeces. Distribution of the excreted toxin in faeces and urine was 87% and 13%, respectively. Our examinations have applied to the unchanged chemical form of FB_1 only. It must be noted that the actual elimination would have presumably been much higher than that if the metabolised chemical forms had also been taken into consideration. Accordingly, it was supposed that the major part of the toxin was excreted in a partly or totally hydrolysed form.

In this experiment, absorption, distribution and elimination of fumonisin B_1 and its metabolites were determined in pigs that were fed a ration containing 45 mg FB_1 kg^{-1} during 10 days.

Materials and methods

Experimental design and animals

Sixteen weaned barrows (Hungarian Large White) from the age of 8 weeks, 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 = 6). The animals were placed in metabolic cages for the trial. The room temperature (20 ^{o}C) was controlled according to 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 experimental animals were fed a basal diet 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; ingredients: wheat 30%, barley 20%, corn 15%, soy-bean 10%, concentrate 15%). A *Fusarium verticillioides* (MRC 826) fungal culture (1%) and Cr_2O_3 (0.5%) was mixed into the feed of experimental animals, so as to provide a Cr-labelled, fumonisin-contaminated (45 mg/kg FB_1; 8.6 mg/kg FB_2; 4.6 mg/kg FB_3) feed. Fumonisins were produced by a locally developed method and with the application of *Fusarium verticillioides* strain MRC 826 (Fodor et al., 2006b). The homogenized fungal culture contained FB_1, FB_2 and FB_3 at a concentration of 5645 mg kg^{-1}, 1083 mg kg^{-1} and 581 mg kg^{-1}, respectively.

After a five-day adaptation period, according to the method of Tossenberger et al. (2000), a special T-cannula was implanted into the distal part of the ileum, before the ileocaecal valve, in order to determine the absorption of FB_1 from the feed marked by Cr_2O_3. At days 1 and 10 of the intoxication period, and 10 days after the withdrawal of the contaminated diet animals were weighed and blood samples were taken. From the blood samples lipid peroxidation parameters were measured, as a complementary examination. From the beginning of the experiment to end, total quantity of urine and faeces was collected, in order to determine the toxin content of them. At the end of both of periods, piglets (n=5 treated and 3 control animals) were killed by bleeding after sedation. Gross pathological examinations were performed, organs were weighed and several organs were sampled, in order to determine their toxin content and lipid peroxidation status.

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

Determination of mycotoxin content in feeds and fungal cultures

The mycotoxin content of the control and the experimental feed was checked. T-2 mycotoxin was determined with GC-MS (LOD: 0.01 mg kg^{-1}), while zearalenone (LOD: 0.01 mg kg^{-1}), DON (LOD: 0.05 mg kg^{-1}) and ochratoxin A (LOD: 0.1 µg kg^{-1}) by a HPLC system.

The determination of FB_1, FB_2 and FB_3 content from the culture and feed samples was confirmed also by a HPLC fluorescence detection method based upon derivatisation with o-phthaldialdehyde (OPA), as described earlier (Fodor et al., 2006a).

The diet fed to the control group did not contain detectable amounts of mycotoxin.

Lipid peroxidation examinations

For the determination of lipid peroxidation, the samples of blood and organs were stored at –82 °C until analysis. Lipid peroxidation was determined by the quantification of malondialdehyde (MDA) levels with 2-thiobarbituric acid method in blood plasma (Placer et al., 1966), liver, lung, brain, kidney and spleen (Mihara et al., 1980). Among the small molecular weight antioxidants, the amount of reduced glutathione (Sedlak and Lindsay, 1968) was measured in blood plasma, and red blood cell haemolysate and in the above-mentioned organs.

Determination of fumonisin absorption and excretion

For the investigation of absorption of FB_1, weaned barrows implanted with a special T-cannula (PVTC) into the distal part of the ileum were used. The operation was carried out 7 days before toxin feeding. In the intoxication period (for 10 days) chymus samples were

collected twice a day (2-3 hrs after feed consumption) from every animal. Based on the rate of FB_1 and Cr_2O_3 in the feed, the absorption degree of the fumonisin was quantifiable from the chymus toxin content. In the experimental diet the concentration of Cr and FB_1 was 3.84 g kg^{-1} and 45 mg kg^{-1}, respectively. During the calculation of FB_1 absorption (as calculated from the Cr-FB_1 ratio in the feed) the 85:1 of Cr:FB_1 ratio was taken as a basis. Therefore, the absorption was calculated (%) based on deviation from the rate of 85:1.

The preparation of the samples was carried out by the method of Christian and Coup (1954), and for the determination of the chymus Cr content atomabsorption method (Atomabsorption Spectrophotometer, AA-6701F Shimadzu) was used. For the atomizing, acetylene-air flame was applied. From day 2, during the calculation it was corrected with the toxin amount excreted back into the intestine by bile (literature data; Dantzer et al., 1999; biliary excretion of FB_1: 1.4% of the dose at 4 h after dosing). It was also taken the recovery rate of Cr (at the end of the ileum; Cr recovery: 98.6%; Köhler, 1993) into consideration.

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

Analysis of the fumonisin content of organs, faeces, urine and chymus

The amount of toxins detectable in different organs and tissues including the liver, lungs, kidney, brain, spleen, the *m. longissimus dorsi* and *m. psoas major* muscles, and abdominal and subcutaneous fat was determined. Furthermore, the toxin content of faecal, urine and chymus samples was measured.

Quantification and identification of intact fumonisin B_1 and fumonisin B_2 and metabolites of FB_1, namely aminopentol and partially hydrolysed fumonisin B_1 was carried out using LC (PerkinElmer, Series 200; USA)-MS (API 3200 LC/MS/MS System; Applied Biosystems, USA). In the absence of analytic standard for FB_2 metabolites, the absorption of FB_2 was not determined. The determination of tissues' and urines toxin content is equal of the described in the method of Fodor et al. (2006a).

Non-dried and non-lyophilized faeces (2 g) and chymus (5 g) samples were extracted by a mixture of 0.1 M ethylenediaminetetraacetate (EDTA) and methanol (8ml; 3/1 v/v) for 60 min. The samples were filtrated through a fiberglass filter (Whatman GF A, Dassel, Germany) after centrifugation (2000 g; 3 min). An aliquot of the filtrate was analysed.

Similarly to our earlier experiment (Fodor et al., 2006a), pure FB_1 and FB_2 (Sigma, Schnelldorf, Germany) were used for the determination. $PHFB_1$ (partially hydrolysed FB_1) standard was prepared by Stephen M. Poling (U.S.A.), according to the method of Poling and Plattner (1999). This standard was an equilibrium mixture of the two partially hydrolyzed FB_1 forms. ($PHFB_1$a - TCA group at the C-15 hydroxy group (hydrolysis at C-14); $PHFB_1$b - TCA group at the C-14 (i.e. hydrolysis at C15)). AP_1 standard for the analysis was made by the

method of Pagliuca et al. (2005). Every standard was diluted with a mixture of ACN/H_2O (1:1). Table 1 shows the validation data for the FB_1 and its metabolites, and FB_2.

Taking the low feed concentrations of FB_3 into account, as compared to the other FB forms, and its presence supposedly under the LOD value, these parameters were not aimed to determine.

[Insert Table 1 about here]

Determination of FB_1 conversion into metabolites

Based on the molecular weights of the fumonisin B_1 compounds (fumonisin B_1 (α): 721 g/mol; aminopentol (β): 405 g/mol; aminopolyols (γ): 563 g/mol) FB_1 conversion into its metabolites was calculated, as described below:

the fumonisin B_1 – aminopentol conversion:

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

the fumonisin B_1 – partially hydrolyzed FB_1 conversion:

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

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

Statistical analysis

The entire measurement dataset was analysed statistically. Correlation analysis ($P \leq 0.05$) was performed to determine the correlation between the concentration of FB_1 and $PHFB_1$ or AP_1 in the faeces samples. Data processing and the mathematical-statistical calculations were

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

Results and Discussion

Clinical signs and findings of the pathomorphological examinations

Pigs consumed 36.6±6.5 mg FB_1 per day did not show clinical signs of disease and their feed consumption was balanced throughout the experiment. At the first examination (after the 10-day long toxin exposure) pulmonary oedema, the typical disease entity caused by fumonisin-contaminated feed, developed in all of the piglets (n=5) as a result of the toxin dose applied. In the pleural cavity of animals a small volume (2–7 ml) of slightly yellowish fluid with clotting characteristics could be found. Of the other organs examined, beside the pathological change of the liver, the heart, and the kidneys, the spleen showed hyperplasia. 10 days after the end of the toxin feeding, in case of 4 animals 8-9 ml fluid was distinctly visible in the pericardium. The pulmonary oedema was estimated as minor degree.

Antioxidant results

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

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

[Insert Figure 1 about here]

Summarized, fumonisin in the applied dose did not cause the production of peroxyl radicals in significant measure in these tissues but in bood (plasma and haemolysed RBC) it resulted reduction in the second line of the antioxidant system, namely in the reduced glutathione. This hypothesis is supported by the findings that fumonisin B_1 induces oxidative stress in human, rat and mouse neural cell cultures. Decreased GSH levels, indicative of lipid peroxidation and necrotic cell death were observed in all cell lines after incubation with FB_1 (Stockmann et al., 2004).

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

Absorption of fumonisin B_1

Table 2 shows the results of the calculated daily absorption (per animal) of FB_1 in the 10-day period. By eighty individual data (taking the molecular weight of FB_1, $PHFB_1$ and AP_1 into account) it was established that the mean accumulative absorption rate of all animals tested for intact fumonisin B_1 and its metabolites formed in the small intestine (till the end of the ileum) is 4%. There was no significant correlation between the daily fumonisin intake and the absorption of the toxin. Until now, about the exact absorption of FB_1 only computed data were available; this is the first result from an absorption measurement.

[Insert Table 2 about here]

Following gavage dosing to vervet monkeys, only small amounts of FB_1 were absorbed, and after 24 hr major organs retained little of the radiolabel, with the liver retaining the most (Shephard et al., 1995). The p.o bioavailability of FB_1 was 3.5% after single doses of 10 mg

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

In the chymus, the FB_1 conversion into aminopentol and partially hydrolysed FB_1 was 1% and 3.9%, respectively (data not shown). Accordingly, the biotransformation (metabolism) of fumonisin B_1 was proven to happen already in the small intestine; the absorption of metabolites (mainly of the aminopentol) might also occur.

No data have so far been reported on the FB_1 metabolite of small intestinal content. In the experiment of Shephard et al (1995), at the time of sacrifice, the stomachs and small intestines of the monkeys were empty, so the scheme of chymus sampling was not successful. All bile samples failed to show the presence of any hydrolysis products in the cited study. Based on this fact, Shephard et al. supposed already in 1995 that the hydrolysis process occurs only in the gut and not in the liver.

Fumonisin content of the organs examined

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

[Insert Table 3 about here]

In the investigated tissues 50% of the recovered FB_1 was intact (Table 3). FB_1 conversion into aminopentol and partially hydrolysed FB_1 was 30% and 20%, respectively. In most of the

organs detectable amounts of FB_1 (50%) and its metabolite, aminopentol (50%) were measured even 10 days after the dosage of the non-contaminated feed.

Excretion of fumonisins

During the 10-day toxin-feeding period, the daily toxin intake of the animals was 36.6 ±6.5 mg (2.2 mg kg^{-1} bw.) in average. In the first five days after begining of the toxin feeding, the FB_1 concentration of the faecal content increased continuously, and then from the 5th day to the 10th day it was about 55 µg g^{-1} (Figure 2). There was no change in the aminopentol concentration after the 3rd day, it was measured in standard but low (6 µg g^{-1}) concentration.

[Insert Figure 2 about here]

Taking the molecular weight of the compounds into account, it was established that during the continuous toxin exposure (in 10 days) 59% of the total fumonisin B_1 compounds recovered in the faeces were determined as partially (47%) or totally (12%) hydrolysed metabolites. Previous toxicokinetic studies on vervet monkeys, based on iv and oral dosing of FB_1, similarly demonstrated the rapid elimination of FB_1 in the faeces (Shephard et al., 1995). The extent of hydrolysis varied considerably, although the main product was the partially hydrolysed form (approximately 1/3 of total fumonisins), with very small recovered amounts of the fully hydrolysed (aminopentol) moiety.

Investigating the period between the 5th and 10th day, and having regard to all fumonisins, 72% (27.3 mg) of FB_1 taken up was recovered daily in the faeces. Between the days 5 and 10, a slower but constant increase was noted in the daily-excreted amount, suggesting a balance between the FB_1 intake and its elimination.

During the time of toxin exposure a relatively close correlation ($r = -0.4$, $P<0.05$) was found between the concentration of FB_1 and $PHFB_1$ in the faeces samples, while there was no statistically significant correlation between the concentration of FB_1 and AP_1.

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

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

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

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

Conclusion

In our first *in vivo* experiment (Fodor et al., 2006a) on the toxin elimination, by the determination of the intact FB_1 moieties, a strongly negative balance was found in the total toxin amount, when taking the moieties excreted in the urine and faeces into account. This was, at least in part, the basis of the present experiment, where the most important and frequent metabolites were also determined.

From the trials on animals, we could draw a conclusion that the intestinal microbiota of pigs is able to transform the intact FB_1 to a similarly toxic substance (partially hydrolysed FB_1) or a more toxic metabolite (aminopentol). As a general principle, from the two metabolites, partially hydrolysed FB_1 has priority during the metabolic process. The conversion of FB_1 to AP_1 is notable even despite of its little amount, because this new compound means a new risk from the viewpoint of animal- and human health as well, taking into account that aminopentol is hydrophobic molecule (with a more effective absorption) and that its further derivative (N-palmitoyl-aminopentol) appears to be tenfold toxic than the original FB_1 (Humpf et al., 1998). On the basis of the calculated accumulative absorption rate (maximally 4%) it is not clear, which metabolites shaped exactly this value. From the point of view of human exposure, special attention should be paid to AP_1 appeared in edible tissues even 10 days after the dosage of the non-contaminated feed because there is a lack of information about the effect of this metabolite consumed in low concentration but for a long term.

AP_1 was less potent than FB_1 as an inhibitor of ceramide synthase *in vitro* (Merrill et al., 1993). A feeding study in which rats were fed corn containing 50 ppm of FB_1 or 10 ppm AP_1 resulted in roughly equal toxicity signs in both groups, indicating that AP_1 could be more

toxic than FB_1 itself (Hendrich et al. 1993). AP_1 shows also cancer-promoting activity in liver (Hendrich et al. 1993). Removing the tricarballylic acids, AP_1 becomes not only an inhibitor but also a substrate for acylation by ceramide synthase. In the presence of palmitoyl-CoA AP_1 is also acylated to form N-palmitoyl-AP_1 (PAP_1), which is a more potent inhibitor of ceramide synthase (Humpf et al. 1998). Thus, if FB_1 is efficiently metabolized to aminopentol, the above mechanism may lead to metabolic activation.

Further investigations are reasoned to clarify the dose-dependent metabolism and absorption of fumonisin B_1.

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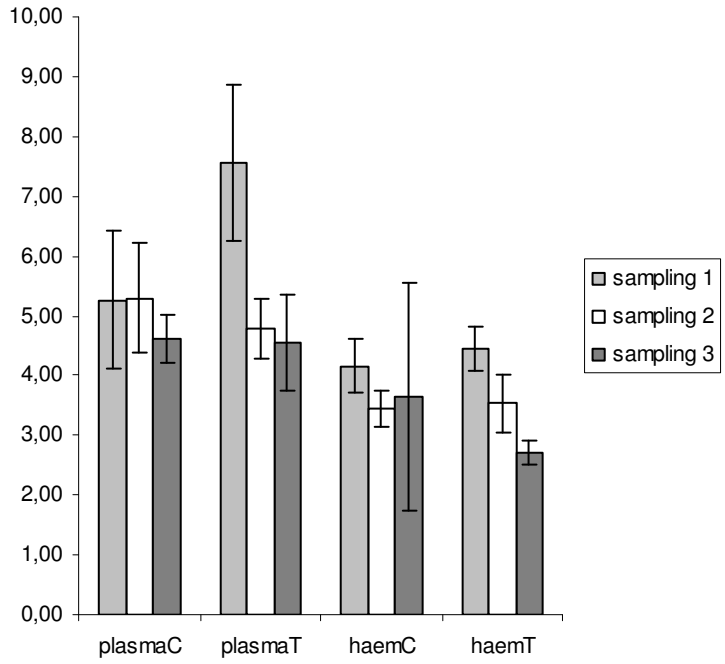

Figure 1. The amount of reduced glutathione (GSH) in blood plasma (plasma) and red blood cell haemolysate (haem) samples (C=contol group; T=group fed contaminated feed)

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)

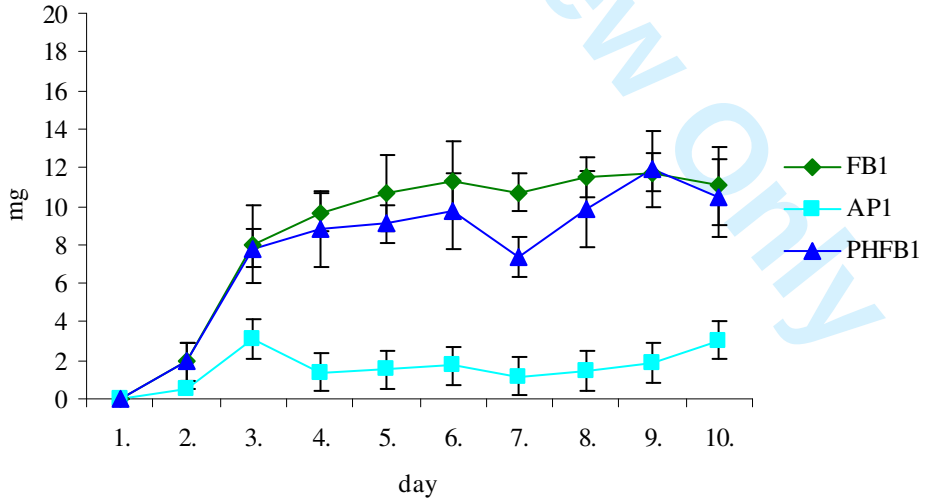

Figure 2. Amount of fumonisins (mg) recovered in faeces during the 10-day long exposure (mean±S.E.; n=10)

Table 1. Validation data of the analysis

Sample		Recovery ±SD[a] (%) (n=5)	LOQ[b] (μg kg^{-1}) (n=5)	LOD[c] (μg kg^{-1}) (n =5)
Faeces, chymus	FB_1	140.0±14.7	100	38.6
	FB_2	107.2±11.7	1000	155.1
	AP_1	123.9±19.2	100	56.0
	$PHFB_1$	106.3±16.2	100	35.6
Urine	FB_1	98.2±11.0	10	1.2
	FB_2	101.3±9.3	10	3.6
	AP_1	104.7±14.0	10	1.9
	$PHFB_1$	101.7±11.8	10	1.2
Lung	FB_1	92.1±4.2	10	1.4
	FB_2	84.1±12.5	10	2.2
	AP_1	98.1±9.1	10	1.3
	$PHFB_1$	67.9±13.3	10	0.9
Liver	FB_1	90.3±3.9	10	1.2
	FB_2	44.1±2.5	10	2.1
	AP_1	93.6±19.6	10	1.4
	$PHFB_1$	47.9±6.0	10	0.8
Kidney	FB_1	60.3±13.3	1	0.9
	FB_2	54.7±1.4	10	1.4
	AP_1	115.2±8.2	1	0.8
	$PHFB_1$	47.2±5.7	1	0.8
Spleen	FB_1	90.2±4.7	10	1.0
	FB_2	72.2±3.7	10	1.3
	AP_1	86.7±8.0	1	0.7
	$PHFB_1$	65.7±5.0	1	0.5
Brain	FB_1	92.6±12.6	1	0.6
	FB_2	91.08±6.8	10	2.3
	AP_1	97.1±15.9	1	0.8
	$PHFB_1$	69.9±10.2	1	0.4
Muscle (m.l.d., m.p.m.)	FB_1	87.5±11.0	10	0.6
	FB_2	60.4±7.2	10	1.2
	AP_1	91.3±8.6	10	0.6
	$PHFB_1$	84.3±15.0	10	0.4
Fat (subcutaneous, abdominal)	FB_1	84.4±4.2	1	0.7
	FB_2	35.1±2.7	100	3.9
	AP_1	109.0±20.1	1	0.6
	$PHFB_1$	89.2±19.9	1	0.6

[a] *standard deviation*
[b] *limit of quantification*
[c] *limit of detection*

Table 2. Accumulative absorption of the fumonisin B_1 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 FB_1 derivatives in the organs and the conversion of the FB_1 molecule into metabolites

Organs/Tissues		**Fumonisins' concentration (mean ±SE; µg kg^{-1})* and the FB_1 conversion (%) into the separate metabolite**					
		Sampling 1 (n=5)			**Sampling 2** (n=5)		
		mean	±SE	%	mean	±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]	-	
Sleep	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. longissi-mus 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