Effect of different dietary fumonisin B₁ exposure on the toxin content of porcine tissues

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ABSTRACT

The time and dose-dependent effect of fumonisin B₁ (FB₁) exposure on the tissue toxin concentrations was examined in 36 weaned pigs. Treated animals were fed an experimental diet supplemented with Fusarium verticillioides culture, leading to FB₁ daily intake values of 50 mg/animal (n=10) in a 22 day treatment (T50), 100 mg/animal (n=7) for 5 days (T100s, short exposure) and 100 mg/animal (n=7) for 10 days (T100l, long exposure), respectively. For each experimental group, 4 control (C) animals were used. At the end of trial, the FB₁ content of lung, liver, bile, kidney, brain, spleen, pancreas, heart, muscle and fat samples was determined by LC-MS. Pulmonary oedema developed in all piglets as a result of the toxin dose applied. Highest FB₁ concentrations were found in the liver, kidney, lung and spleen, in all treatments. The muscle and adipose tissues (i.e. the meat) did not contain considerable amounts of fumonisin B₁. In the organs of the animals fed 100 mg FB₁ per day, significantly higher FB₁ levels were measured. An exponential function was descriptive for the measured FB₁ data of some organs. The FB₁ content was found to be dependent on the average daily intake, except in case of the liver.

Key words: Fumonisin B₁, Pig, Mycotoxicosis, Feed contaminant

RIASSUNTO

L’effetto della dose e del tempo di esposizione a fumonisina B₁ (FB₁) è stata valutata su 36 lattonzoli sottoposti a concentrazioni tossiche di micotossina. I suinetti sono stati divisi in 4 gruppi sperimentali di cui quello di controllo (n=12 lattonzoli) era alimentato con la dieta di base e quelli trattati ricevevano la stessa razione integrata con livelli crescenti di una coltura di Fusarium verticillioides che consentiva l’assunzione di: 50 mg/lattonzolo/giorno (n=10) di FB₁ per un periodo di 22 giorni (trattamento T50); 100 mg/lattonzolo/giorno (n=7) per 5 giorni (trattamento T100s, esposizione breve) e 100 mg/lattonzolo/giorno (n=7) per 10 giorni (trattamento T100l, esposizione prolungata). Al termine della prova campioni di polmone, fegato, bile, reni, cervello, midollo, pancreas, cuore, tessuto muscolare e grasso sono stati analizzati per il contenuto di FB₁ con l’aiuto di LC-MS. Tutti i suinetti trattati con FB₁ hanno sviluppato edema polmonare come conseguenza della dose tossica impiegata. In tutti i soggetti trattati, gli organi che hanno evidenziato le maggiori concentrazioni di micotossina sono stati il fegato, i reni e i polmoni mentre i tessuti muscolari e adiposi non hanno evidenziato rilevanti contaminazioni da fumonisina B₁. Gli organi dei lattonzoli alimentati con 100 mg di FB₁ per giorno hanno evidenziato le concentrazioni significativamente maggiori di micotossina. All’aumentare della dose assunta con la dieta, il contenuto di FB₁ in alcuni organi ha avuto un andamento di tipo esponenziale. La concentrazione di questa mico-
Introduction

Fumonisins were first isolated in 1988 from a Fusarium moniliforme (F. verticillioides) maize-grown culture of the strain MRC 826 (Gelderblom et al., 1988; Cawood et al., 1991), and their molecular structure was also determined (Bezuidenhout et al., 1988). From the viewpoint of toxin exposure, special attention should be paid to fumonisin B₁ (FB₁), being responsible for the development of oesophageal cancer (Marasas et al., 1988) in humans, leukoencephalomalacia in horses (Marasas et al., 1988) pulmonary edema in pigs (Fazekas, 1998), hepatic cancer in rats and nephrotoxic and neurotoxic effects in several animal species. Therefore after aflatoxins and ochratoxin, fumonisins are regarded as potentially the most dangerous mycotoxins, mainly with respect to carcinogenic character in humans (IARC, 1993).

The chemical structure of fumonisins is highly similar to that of sphingolipids, therefore they inhibit the biosynthesis of sphingolipids by blocking the sphinganine-N-acyltransferase enzyme action. Alterations induced in the cellular function and morphology can be attributed on one hand to the lack of sphingolipids, and on the other to the accumulation of cytotoxic metabolites such as sphinganine (Kim et al., 1991).

The primary source of FB₁ toxicosis is fumonisin infected maize. The analysis of numerous further food items and raw materials in several countries has revealed that those, listed below, do not mean any health risk from the viewpoint of toxin exposure. Thus, the toxin could be detected from beef (2070 ng/g FB₁ in the liver, 97.3 ng/g FB₁ in the muscle, and 23.4 ng/g FB₁ in the kidney) but only after prolonged exposure to extremely high FB (400 ppm FB₁ and 130 ppm FB₂ for 30 days) doses (Smith and Thakur, 1996). In the United States, fungal culture material containing fumonisin B₁ (75 ppm) was mixed into the total diet and fed for 14 days to two midlactation Jersey cows to determine if fumonisins are excreted in milk. Fumonisins were not detected in any of the milk samples by two analytical laboratories using methods with a sensitivity of 5 ng/ml (Richard et al., 1996). After intravenous administration of ¹⁴C-FB₁ to pigs, the accumulation of the toxin could not be demonstrated in the pork, while the liver and the kidney (1076 and 486 ng FB₁ and/or metabolites per g tissue, respectively) were found to be sites of accumulation (Prelusky et al., 1994). In laying hens, the toxin does not form significant residue amounts neither in the different tissues nor in the eggs (Vudathala et al., 1994).

In order to determine fumonisin B₁ residue formation in porcine tissues, the short- and long-time effects of FB₁ fed in different doses were examined in weaned pigs.

Material and methods

In the experiment, 36 weaned (24 treated and 12 control) castrated pigs of the same genotype, weighing 13±1.2 kg, were used. Treated animals were divided into three experimental groups: in T50 (n=10) 50 mg FB₁/animal/day for 22 days, in T100s (n=7) 100 mg FB₁/animal/day for 5 days and in T100l (n=7) 100 mg FB₁/animal/day for 10 days was fed. For each experimental group, 4 control (C) animals were used.

The experimental animals were fed a basal ration of a composition corresponding to their age (187 g/kg crude protein, 12.8 MJ/kg metabolisable energy, 13.1 g/kg lysine). After a 5-day adaptation period, the Fusarium verticillioides fungal culture containing a known amount of FB₁ was added to the treated animals’ diet. The fungal culture was produced at the Veterinary Institute of Debrecen according to the method of Fazekas (1998). Control animals were fed a toxin-free diet (T-2, zearalenone, deoxynivalenol and ochratoxin A toxins were not detectable in the diet). The mycotoxin content of the diet was determined by HPLC-system using fluorescence detection, according to the method of Fazekas et al. (1996).

All animals were kept individually during the trial. Pigs were weighed weekly in T50 and on the
1st, 5th and 11th day of the experiment in T100s and T100l. Their clinical status was continuously monitored. Feed was given twice a day, in two equal portions, and the amount of refusals was measured back. Drinking water was available ad libitum via automatic drinkers.

One animal was discarded from group T100s due to insufficient health status that was, however, independent from the toxin exposure.

At the end of the 5, 10 and 22 day-trials pigs were exsanguinated (i.e. on the 6th, 11th and 23th day) after tranquillisation (Vetranquil 1% inj., Phylaxia-Sanofi, Budapest, Hungary) and necropsied. Gross pathological examination was performed and the lungs, heart, liver, kidneys, spleen, brain and pancreas were weighed. Lung, liver, bile, kidney, brain, spleen, pancreas, heart, muscle (Longissimus dorsi, Biceps femoris, Psoas major), subcutaneous and abdominal fat were sampled for FB1 measurement. The analysis was performed at Institute of Animal Hygiene of the Technical University of Munich, by the HPLC method (Waters 2690 Separations Module, Milford, MA) - MS (VG Platform 2) of Meyer et al. (2003).

Results and discussion

In case of 50 mg/animal/day FB1 dose (T50) no clinical disease signs were found on the pigs. The feed consumption was balanced throughout the experiment and no feed refusal (indicative of toxin effect) was observed. In contrast, animals in groups T100s and T100l became depressed, lost appetite, and their feed intake decreased on the 5th-6th day. Pigs showed severe dyspnoea while the mucous membranes indicated signs of cyanosis. Clinical symptoms rapidly developed and pulmonary oedema led to death within 12-24 hours after the first signs. One and two pigs of T100l group died on the 5th and 6th day of the experiment, respectively. Two more animals of T100l group died on the 8th day.

Pulmonary oedema, the typical disease entity caused by FB1 toxin, developed in all piglets, as a result of the doses applied. The thoracic cavity of the pigs contained less or higher amount (15-390 ml) of yellow exudates inclined to coagulation, but by animals in T100s and T50 groups, the presence of the fluid was observed in more cases and in higher amounts. The liver, the heart and the kidneys showed pathological alterations. The most remarkable pathological findings included e.g. enlarged, friable, pale, yellowish liver, pale kidney with spot-like necrotic areas, and small volume of pericardial fluid, containing lumps. The trachea and the bronchi contained a white and frothy substance. Haemorrhagic infiltration of the peribronchial lymph nodes was also found, but only in the most serious cases (T100l). Significant difference was not found between the experimental groups and the control in organ weights.

The average total FB1 intake in T100s group was 403.8 mg, i.e. 33.1 ± 3 mg/kg BW (Table 1). During the 22-day feeding period (T50), the mean toxin intake of the animals was 1091.2 mg, while the total toxin uptake was 54.6 ± 3 mg/kg of BW. The highest FB1 concentrations were found in the liver and in the kidneys (Figure 1). Substantial toxin amounts were measured in the myocardium and spleen, while minimal FB1 concentrations were measured in the lungs.

The total FB1 intake of the two surviving animals in the T100l group was on average 742 mg (53 mg/kg BW) in the whole experiment. In both groups (T100s and T100l), the toxin concentrations of the organs showed very large variations. Particularly high levels were measured in the kidney, the liver, the lung and the spleen (Figure 1). Muscle and fat samples showed negligible contamination (59.8 ±5.6 and 37.4 ±4.8 ng/g, respectively).

These findings show good agreement with the results obtained in the experiment of Prelusky et al. (1996), according to which the highest toxin levels could be measured in the liver and kidneys of pigs fed a diet containing 2–3 ppm radiolabelled FB1 for a period of 24 days.

Individual differences of the toxin content in the organs could be attributed to numerous conditions. On one hand, the cumulative uptake of FB1 varied between the animals. Due to the rapid clearance of FB1 in pigs (Prelusky et al. 1996), the animals with a daily dose of total appointed FB1 till death had explicitly higher residues in the tissues than the pigs consuming no or little mycotoxin in the day before exitus. Thus, it is more likely that the tissue concentration is connected with the
mycotoxin uptake directly prior to the death. Variations in the individual absorption efficacy and metabolism may also have contributed to the differences of detectable residues.

The liver, kidney, spleen and lung were the organs with the highest toxin contents; according to the FB$_1$ intake, significant differences ($P \leq 0.05$) were found among them in groups T100$_s$, T100$_l$ and T50 (Figure 1). Obviously, significantly higher levels were measured in the organs of the group T100$_s$.

No detectable levels of fumonisin B$_1$ were measured in the organs of the control pigs.

Comparing data of table 1. and figure 1., a negative correlation was supposed between the absolute toxin intake and the toxin content of the organs, i.e. the more toxin got into the organism the less was accumulated in the organs. However, it must be noted that in T100$_s$ and T100$_l$ groups the toxin was consumed only for a short period, accordingly, the average daily intake per animal and per body weight was higher than in T50, while the total consumed FB$_1$ amount was less. Accordingly, from the viewpoint of toxin exposure not the absolute intake during a period is determinant, but the daily toxin amount, what is incorporated by the organism directly before the slaughter. This is supported by the close positive correlations found between the average daily intake (Table 1) and the toxin content of some organs (Table 2).

Figure 2. shows the relationship between the average daily toxin intake and toxin-concentration of the kidney, the liver and the muscle Longissimus dorsi. In case of the kidney and the muscle, an exponential curve was fit to the data. Close positive correlations among variable-pairs, moreover, a relatively well-adapting exponential

| Table 1. Calculated parameters of the toxin consumption. |
|---------------------------------------------------------|
| Duration of treatment | T50 | T100$_s$ | T100$_l$ |
| Animals | n. | 10 | 6 | 2 |
| Average live weight at the end of trial | kg | 20.0 | 12.2 | 14.0 |
| Total intake/animal | mg | 1091.2 | 403.8 | 742 |
| Total intake/body weight | mg/kg BW | 54.6 | 33.1 | 53 |
| Average daily intake/animal | mg/d | 49.6 | 80.8 | 74.2 |
| Average daily intake/body weight | mg/kg BW | 2.5 | 6.6 | 5.3 |

| Table 2. Correlations between the average daily FB1 intake and the toxin content of some organs. |
|---------------------------------------------------------------|
| Organ | No. of animals with detectable toxin concentration | Correlation (r) | Significance (P) |
|------|--------------------------------------------------|----------------|-----------------|
| Liver | 18 | 0.674 | 0.002 |
| Kidney | 18 | 0.671 | 0.002 |
| Longissimus dorsi | 11 | 0.632 | 0.037 |
function was found, with high “R” values. For the liver – presumably due to a relative high standard deviation (mean: 142.5 ng/g; S.D.: 74.6) – a linear relationship (Figure 2b) could be better adapted.

Conclusions

In all groups the highest concentrations of FB1 were found in the liver, kidney, spleen and lung. According to the FB1 intake, significant differences (P≤0.05) were found among them in groups T50, T100s and T100l. The muscle and adipose tissue did not contain considerable amounts of fumonisin B1. An exponential function may adapt to data of some organs’ FB1 content being dependent on the average daily intake, except the liver (linear relationship). Because the toxin does not cumulate in the organism, not the absolute toxin amount consumed, but rather the daily intake seems to be determinant from the viewpoint of toxin exposure.

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