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Determination of fumonisin B₁ levels in body fluids and hair from piglets fed fumonisin B₁-contaminated diets

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Abstract

The levels of fumonisin B₁ (FB₁) residues in plasma, urine, feces and hair from 24 piglets fed FB₁-contaminated diets containing 3.1, 6.1 or 9.0 μg FB₁.g⁻¹ for 28 days were determined using liquid chromatography coupled to mass spectrometry (LC-MS/MS). The levels of FB₁ in plasma, urine, feces and pooled hair (*n* = 3) samples varied from 0.15-1.08 μg.L⁻¹, 16.09-75.01 μg.L⁻¹, 1.87-13.89 μg.g⁻¹ and 2.08-8.09 ng.g⁻¹, respectively. Significant correlations (*r* = 0.808-0.885; *P* < 0.001; *N* = 18) were found between FB₁ intake and plasma FB₁ on days 7, 14, 21 and 28. However, urinary FB₁ correlated with FB₁ intake only on days 7 and 14 (*r* = 0.561-572; *P* = 0.02; *N* = 18). A significant correlation (*r* = 0.509; *P* = 0.02; *N* = 24) was also found for the first time between FB₁ in hair samples and FB₁ intake. Plasma and urinary FB₁ are good biomarkers of early exposure of pigs to low dietary FB₁ levels, although plasma is recommended to assess prolonged exposure (> 14 days). The possibility to evaluate hair as a biomarker of fumonisin exposure was established, although further studies are needed to provide physiologically based toxicokinetics of residual FB₁ in the pig hair.

Keywords: FB₁; plasma; urine; hair; biomarker; exposure assessment.

1. Introduction

Fumonisin B₁ (FB₁) is a toxic metabolite produced by strains of *Fusarium verticillioides* and *F. proliferatum* during growth on several food and feed materials (Diaz 2005). FB₁ contamination in food has been associated with human esophageal cancer, being classified in group 2B as possible carcinogen in humans by the International Agency for Research on Cancer (2002). Furthermore, the dietary exposure to FB₁ has been correlated with an increased incidence of human neural tube defects along the Texas–Mexico border (Missmer et al., 2006). Since their identification in 1988, FB₁ has been associated with animal diseases such as equine leucoencephalomalacia (ELEM) and porcine pulmonary edema

(PPE). Pigs are extremely sensitive to FB₁ intoxication at levels usually above 30 µg.g⁻¹ (Ahangarkani et al. 2014), manifestations in addition to PPE include lower weight gain, poor feed conversion, reduced reproductive efficiency (Scott, 2012), intestinal damage (Loiseau et al. 2007; Greneir et al 2012) and increased susceptibility to infections (Halloy et al. 2005; Devriendt et al 2009). As a preventive measure to avoid the adverse effects of FB₁ in pigs, the United States and the European Union have established tolerance limits of 20 µg.g⁻¹ for corn grain and 5 µg.g⁻¹ for complete feed, respectively (Oliveira et al. 2014).

The oral bioavailability of FB₁ in several animal species tested is lower than 6% (European Community 2006), with an estimated value of 3.5% for pigs (Martinez-Larrañaga et al. 1999). In pigs, the absorbed FB₁ is rapidly distributed and eliminated from the circulation, and large amounts of unmetabolized FB₁ are recovered in feces (Dilkin et al. 2003). Although little is known about the biotransformation of FB₁ by mammalian metabolism, some *in vivo* studies performed on primates (Shephard et al. 1994), ruminants (Rice and Ross 1994) and pigs (Fodor et al. 2008) revealed that the ester moiety of FB₁ was hydrolysed in the intestine, forming aminopentol and a partially hydrolysed FB₁ compound. Moreover, extensive enterohepatic circulation was observed in pigs exposed to high oral doses (500-5,000 µg.kg⁻¹ body weight) of FB₁ (Prelusky et al. 1994; Dilkin et al. 2010). However, there is little information on the effects of chronic exposure to low dietary levels of FB₁ in pigs, which are most probable to be found under natural conditions.

In recent years, animal and human exposure to dietary mycotoxins has been assessed using biomarkers in biological matrices, aimed at providing a more accurate assessment of exposure at the individual level. A biomarker of exposure refers to the quantification of the specific compound, its metabolite(s) or interaction products in a body compartment or fluid, which indicates the presence and magnitude of exposure to the compound (Jager et al. 2011). Analytical methods based on liquid chromatography coupled to mass spectrometry (LC-

MS/MS) have been developed for determination of biomarkers of several mycotoxins including FB₁ in pig plasma (Devreese et al. 2012) and urine (Gambacorta et al. 2013). In laying hens, plasma half-life of FB₁ was short and the bioavailability was only 0.5 % (Vudathala et al. 1994). Studies in several animal species have shown that excretion of orally consumed FB₁ in urine is generally low, typically less than 2% (Shephard et al., 2007). Additionally, experimental studies with nonhuman primates and rats have demonstrated that FB₁ residues can be quantified in hair, hence indicating a potential use of this measurement as a biomarker of exposure (Sewram et al. 2001). Bordin et al. (2015) determined FB₁ levels in human hair from volunteers in Brazil and found low concentrations that correlated with FB₁ probable daily intake values of the individuals evaluated. However, no previous study on FB₁ residues in pig hair has been published. Therefore, the aim of this study was to determine the levels of FB₁ residues in plasma, urine, feces and hair from piglets fed FB₁-contaminated diets, in order to evaluate their suitability as biomarkers of exposure to prolonged, low dietary levels of FB₁.

2. Material and methods

2.1. Animals, diets and experimental design

The experiment with pigs was carried out in accordance with the European Guidelines for the Care and Use of Animals for Research Purposes (Directive N°: TOXCOM/0018/PP IO, Comité d'Éthique de Pharmacologie – Toxicologie de Toulouse – Midi Pyrénées, en matière d'expérimentation animale (Toxicométrie, INRA). Twenty-four 28-day-old weaned castrated male piglets (Pietrain/Duroc/Large-white) were allocated in 8 cages (3 animals per cage) and allowed *ad libitum* access to feed and water. The basal diet (BD) was prepared from wheat, soybean meal and barley, and was formulated to meet or exceed the nutritional requirements of growing pigs as recommended by Grenier et al. (2011). The

animals were acclimatized for 7 days, reaching a mean body weight of 10.0 ± 0.9 kg. After this period, pigs were randomly assigned into 4 experimental groups of 6 pigs each (2 cages with 3 pigs each), which were exposed over 28 days to 4 dietary levels of FB₁, as follows: 0 FB₁ (BD only, control), $3.1 \mu\text{g}\cdot\text{g}^{-1}$ FB₁, $6.1 \mu\text{g}\cdot\text{g}^{-1}$ FB₁ and $9.0 \mu\text{g}\cdot\text{g}^{-1}$ FB₁. The animals were individually weighed at the beginning and at 7 days intervals throughout the experiment. Feed consumption was measured weekly at the cage level, and the value was divided by 3 to obtain the mean individual feed consumption. Individual FB₁ intake was calculated by the following expression: Intake = (concentration of FB₁ in the diet x mean individual feed consumption) / (individual body weight).

Fumonisin was supplied by *F. verticillioides* M-1325 culture material, prepared by the Veterinary Medical Diagnostic Laboratory of the University of Missouri, USA, as reported previously (Weibking et al., 1993). The culture material was previously analyzed for FB₁ and FB₂ by the procedure of Hopmans and Murphy (1993), which revealed concentrations of $1,800 \mu\text{g FB}_1\cdot\text{g}^{-1}$, and $450 \mu\text{g FB}_2\cdot\text{g}^{-1}$. Fumonisin culture material was added to the basal diet in appropriate amounts, so as to obtain the required levels of FB₁ (3.1 , 6.1 and $9.0 \mu\text{g FB}_1/\text{kg}$ diet) at the INRA facilities of Rennes as already described (Meissonnier et al. 2008; Grenier et al. 2011). After preparation of the experimental diets, a 1-kg sample was collected from each treatment and analyzed by a LC-MS/MS method based on procedures as described by Sørensen and Elbaek (2005) in the Laboceia laboratory (Ploufragan, France) to confirm the fumonisin levels. The concentrations of FB₁ in final diet were $3.1 \mu\text{g}\cdot\text{g}^{-1}$, $6.1 \mu\text{g}\cdot\text{g}^{-1}$ and $9.0 \mu\text{g}\cdot\text{g}^{-1}$; and the one of FB₂ $0.6 \mu\text{g}\cdot\text{g}^{-1}$, $2.0 \mu\text{g}\cdot\text{g}^{-1}$ and $3.2 \mu\text{g}\cdot\text{g}^{-1}$. The BD was also analyzed by the same method as described (Sørensen and Elbaek 2005) and had no detectable levels of fumonisins (detection limit: $10 \text{ ng}\cdot\text{g}^{-1}$). Deoxynivalenol, and ochratoxin A were found to be naturally present in the cereals used, resulting in concentrations of 0.025 , $0.006 \text{ m}\cdot\text{kg}^{-1}$ of feed, respectively. All other mycotoxins, including aflatoxins, T-2 toxin, HT-2 toxin and the

ergot alkaloids were below the limit of detection (0.004 mg.kg⁻¹, 0.01 mg.kg⁻¹, 0.01 mg.kg⁻¹ and 0.01 mg.kg⁻¹, respectively).

2.2. Sample collection

Blood, urine, feces and hair samples were collected at 7 days intervals throughout the experiment starting on day 7. Individual blood samples (8.0 mL) were aseptically collected by jugular venipuncture using tubes containing sodium heparin (Vacutainer®, Becton-Dickinson, USA). Plasma samples were obtained after centrifugation of blood at 3,200 x g for 15 min at 4° C, and 4-mL aliquots were transferred to Eppendorf tubes (Meissonnier et al. 2008).

Once a week, animals were placed in metabolic cages to collect urine and feces as previously described (Gambacorta et al. 2013). Briefly, urine samples were collected in glass jars and then transferred into conical test tubes (15 mL), and subsequently separated into aliquots of 1.5 mL. Plasma and urine samples were added with the preservative 2-Bromo-2-nitro-1,3-propanediol (Sigma-Aldrich, Saint Louis, MO, USA), to give a final concentration of 0.9 mg.mL⁻¹, and stored at -80° C. Individual feces samples were collected in plastic bags and dried in Petri dishes in an oven at 50° C for 24 hours. The dried feces were kept in clean plastic bags at room temperature. Hair samples were collected by cutting with an electric hair clipper all the scalp hair available from the skin (approximately 2 cm length), in different areas of nearly 100 cm² on each animal's back and dorsal laterals. Because limited amounts of hair were available due to slow hair growth rate in the experimental pigs, hair samples from 3 pigs in each treatment were combined and stored in plastic bags, in order to obtain a minimum amount required for analytical determinations (2.0 g). Hence each treatment provided 2 pooled hair samples in each time of sample collection. Each plastic bag containing a pooled hair sample had the air inside removed, and then it was folded and finally placed in cartoon boxes. All samples collected from pigs were transported on dry ice to the Laboratory

of Food Mycotoxicology and Microbiology, Pirassununga, SP, Brazil, for fumonisin analyses. Upon arrival, plasma, urine and hair samples were stored at -80°C for a maximum period of 3, 5 and 10 months prior to analysis, respectively. Feces samples were analyzed immediately after arriving in the laboratory. Blank samples of plasma, urine, feces and hair collected from pigs fed BD alone (control), with no detectable levels of FB_1 , were used for the preparation of matrix-matched calibration curves and quality control samples.

2.3. Preparation and analyses of samples

All reagents were of analytical grade and water was purified by deionization (Milli-Q system, Millipore, Bedford, MA, USA). HPLC grade acetonitrile and methanol (JT Baker, Xalostoc, Mexico) were used for chromatographic analyses. PTFE filter units ($0.22\ \mu\text{m}$, Millex®) were obtained from Millipore®. The analytical standard of FB_1 was purchased from Sigma-Aldrich (Saint Louis, MO, USA) and stored at -20°C . An isotopic standard ($^{13}\text{C}_{34}$ - FB_1) was also obtained from Sigma-Aldrich (Saint Louis, MO, USA) at the time of hair analyses. Stock solutions of FB_1 ($100\ \mu\text{g}\cdot\text{mL}^{-1}$), $^{13}\text{C}_{34}$ - FB_1 ($10\ \mu\text{g}\cdot\text{L}^{-1}$) and FB_1 intermediate standard solutions (5, 10 and $1000\ \mu\text{g}\cdot\text{L}^{-1}$) were prepared in acetonitrile/water (50:50, v/v), and stored in amber glass vials at -20°C . Calibration curves were prepared by appropriate dilution of intermediate standard solution to produce working standards at concentrations from 0.04 to $0.40\ \mu\text{g}\cdot\text{L}^{-1}$ for plasma, from 0.1 to $50\ \mu\text{g}\cdot\text{L}^{-1}$ for urine and from 0.04 to $2.60\ \mu\text{g}\cdot\text{g}^{-1}$ for feces analysis. The working standard solutions were used to generate a 5-point standard, matrix matched calibration curve for each type of sample analyzed. These solutions were prepared by serial dilutions from the higher concentration levels in blank plasma, urine and feces extracts ($0.40\ \mu\text{g}\cdot\text{L}^{-1}$, $50\ \mu\text{g}\cdot\text{L}^{-1}$ and $2.6\ \mu\text{g}\cdot\text{g}^{-1}$, respectively). To this end, convenient aliquots from the FB_1 standard solutions were transferred into glass vials, the

solvent was evaporated to dryness under nitrogen and finally reconstituted with 1000 μL of plasma, urine or feces blank extracts.

An in-house analytical method was developed and validated for determination of FB_1 residues in samples of plasma, urine and feces. Plasma samples were thawed at room temperature, aliquoted (250 μL) into test tubes (1.5 mL) containing 250 μL of acetonitrile. The mixture was vortex for 15 s and centrifuged at 3,200 x g for 10 min. Four hundred microliters of the supernatant were diluted with an equal amount of deionized water and filtered through a 0.22 μm membrane. The filtrate was transferred into a vial and reserved for LC-MS/MS analysis. After thawing the urine samples at room temperature, they were vortex-mixed and centrifuged at 3,200 x g for 10 min. One hundred μL of supernatant was transferred to a 1.5-mL vial and 900 μL of acetonitrile:water (25:75, v/v) was added. After vortexing for 15 s, the mixture was filtered through a 0.22 μm membrane and reserved for LC-MS/MS analysis. Dried feces samples were triturated and homogenized. Aliquots (0.5 g) were weighed into test tubes, re-suspended with 5 mL of deionized water and vortexed for 5 s. The mixtures were allowed to stand at room temperature for 30 min, then the tubes were vortexed again for 5 s and finally sonicated for 5 min. The mixtures were centrifuged at 3,200 x g for 5 min., and 50 μL of the supernatants were transferred to another tube containing 1,950 μL of acetonitrile:water (25:75, v/v). The mixtures were filtered through 0.22 μm membranes and reserved for LC-MS/MS analysis.

Extraction and purification of FB_1 in pig hair samples were performed exactly as described by Bordin et al. (2015). This method was chosen for the study because it was previously validated in our laboratory using human hair samples, and because it includes a solid phase extraction (SPE) cleanup step to concentrate the extract solutions and therefore increase the analytical sensitivity. The ability of the analytical method to detect and quantify low residual levels of FB_1 in pig hair was of major importance, considering that the literature

available (Sewram et al. 2001; Sewram et al., 2003; Bordin et al. 2015) indicated that lower levels of FB₁ were expected in hair than in plasma, urine or feces samples. Before SPE, hair samples (0.5 g) were spiked with 200 pg of internal standard by adding 20 µL of [¹³C₃₄]-FB₁ stock solution (10 µg.L⁻¹) to each sample. Hair samples were stirred in ethanol at room temperature for 15 minutes. The specimens were dried at 50° C for 3 hours, cut up with scissors into small pieces and stored in whirl packs at -20° C. Hair samples were extracted with 20 mL of methanol overnight at 45°C. The extract was filtered (Whatman 4) and 15 mL of the extract was taken to dryness, diluted in 20 mL of methanol:water (70:30), an additional 8 mL of water was added and then filtered through Whatman 4 filter. The solution was washed with 5 mL of hexane to remove lipophilic compounds, centrifuged at 3,000 x g for 5 min. and the top hexane layer discarded. The pH was adjusted to 6, and an aliquot of 20 mL of the extract was passed through a SAX cleanup column (Phenomenex, Torrance, CA) that had been previously conditioned with 8 mL of methanol and 8 mL of methanol:water (3:1). The column was washed with 8 mL of methanol:water (3:1), 3 mL of methanol and the fumonisin eluted with 14 mL of methanol:acetic acid 0.5%. The eluent was evaporated to dryness and resuspended in 200 µL of water:acetonitrile (9:1 v/v) for subsequent LC-MS/MS analysis. A 6-point standard calibration curve was prepared at concentration range from 0.05 to 50 µg.L⁻¹, by appropriate dilution of FB₁ working solutions. Each point of the calibration curve contained 200 pg of the internal standard, and the final results were corrected for recovery.

2.4. LC-MS/MS analysis

Analytical procedures for all types of samples (plasma, urine, feces and hair) were carried out using a Waters Acquity I-Class LC-MS/MS system (Waters, Milford, MA, USA) equipped with a BEH C₁₈ column (2.1 × 50 mm, 1.7 µm) and coupled to a Xevo TQ-S mass

spectrometer (Waters, Milford, MA, USA). Data collection and processing were performed using MassLynx software, version 4.1 (Waters, Milford, MA, USA). Gradient elution was employed for plasma, urine and feces samples, with mobile phase composed of water (eluent A) and acetonitrile (eluent B), both containing 0.1% of formic acid with a flow rate of the mobile phase of 0.6 mL.min⁻¹. Initial mobile phase composition was 70% of A. The percentage of B was linearly increased to 95% over 1.0 minute, followed by a hold time of 0.5 minute. Finally, the percentage of B was reduced to 30% and the column re-equilibrated to initial conditions for 0.5 minute. Total run time was 2.0 min. For hair samples, the same mobile phase was employed for elution, but the gradient started with 10% of B, which was linearly increased to 40% B at 1.30 min and 90% B at 1.5 min, followed by a hold time of 1.5 min. The percentage of B was reduced to 10% at 3.1 min, and the column re-equilibrated to initial conditions for 0.9 minute at a flow rate of 0.6 mL.min⁻¹ and a total time of 4 min. For all samples, the column was maintained at 40° C and samples at 15° C during analysis. Five microliters of plasma, urine and hair extracts, and 2 µL of feces extracts, were injected into the chromatographic system. The mass spectrometer was operated in Multiple Reaction Monitoring (MRM) mode using electrospray ionization in the positive ion mode, with a capillary voltage of 0.75 kV, source temperature of 150° C and desolvation temperature of 550° C. Desolvation gas flow and cone gas flow were maintained at 800 L.h⁻¹ and 150 L.h⁻¹, respectively. Quantification and confirmatory MRM transitions for FB₁ were *m/z* 722.5 > 352.1 and 722.5 > 334.0, respectively. A transition of *m/z* 756.6 > 374.4 was used for [¹³C₃₄]-FB₁ detection.

2.5. Method validation for FB₁ analyses in plasma, urine and feces

Accuracy, expressed as relative standard error (RSE), recovery and intra-day precision were assessed by adding FB₁ to blank samples to generate final concentrations of 0.04, 0.10,

0.20 and 0.40 $\mu\text{g}\cdot\text{L}^{-1}$ of FB_1 in plasma, 0.1, 25 and 50 $\mu\text{g}\cdot\text{L}^{-1}$ of FB_1 in urine and 0.04, 0.4 and 2.6 $\mu\text{g}\cdot\text{g}^{-1}$ of FB_1 in feces, as presented in Table 1. Each fortification level was prepared in five independent replicates, and all fortified samples were submitted to analysis as described in sections 2.3 and 2.4. Final concentrations were calculated by duplicate injection of each extract. Inter-day precision was assessed by analyzing each fortified sample in three different days. The limits of detection (LOD) and quantification (LOQ) were calculated based on a signal to noise ratio of 3:1 and 10:1, respectively. Linearity was evaluated by verifying the coefficient of determination (r^2) and back calculated concentrations.

2.6. Statistical analysis

Data on body weight and feed consumption from all treatment groups, as well as FB_1 intake and its respective levels in samples of plasma, urine, feces and hair from groups receiving FB_1 dietary levels of 3.1, 6.1 or 9.0 $\mu\text{g}\cdot\text{g}^{-1}$ at different times were subjected to one-way ANOVA using the General Linear Model procedures of SAS® (SAS Institute, 1992). The means for treatments showing significant differences in the analysis of variance were compared using the Tukey test. The results found in plasma, urine and hair samples in each experimental group fed FB_1 were also used to determine Pearson's Correlation Coefficient (r) between the FB_1 intake and the concentrations of FB_1 in the samples collected at different times. A Student t test, with normal approximation, was used to compare the correlation values (Snedecor and Cochran, 1967). Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Method validation

Table 1 shows the recovery and precision results estimated for FB_1 in blank samples of plasma, urine and feces samples. The LOD values for FB_1 in plasma, urine and feces were

0.014 $\mu\text{g}\cdot\text{L}^{-1}$, 0.040 $\mu\text{g}\cdot\text{L}^{-1}$ and 0.012 $\mu\text{g}\cdot\text{g}^{-1}$, respectively. LOQ values were 0.04 $\mu\text{g}\cdot\text{L}^{-1}$, 0.10 $\mu\text{g}\cdot\text{L}^{-1}$ and 0.04 $\mu\text{g}\cdot\text{g}^{-1}$ for plasma, urine and feces, respectively. Calibration curves showed coefficients of determination higher than 0.99, and back calculated concentrations did not exceed 15% from nominal value. Recoveries ranged from 70-78%, 99-106% and 76-79% for samples of plasma, urine and feces, respectively, and the coefficient of variation ranged from 0.1 to 4.0% for all types of samples. For hair samples, LOD and LOQ values obtained using FB_1 internal standards were 0.06 and 0.02 $\text{ng}\cdot\text{g}^{-1}$, respectively, and coefficients of determination of calibration curves were higher than 0.99.

3.2. Growth performance and FB_1 intake by experimental pigs

Body weight, feed consumption and FB_1 intake of pigs after 7, 14, 21 and 28 days of exposure are presented in Table 2. The body weights of pigs from all treatments increased continuously ($P < 0.05$) from day 7 (13.3-14.0 kg) until day 28 (28.4-32.0 kg), although there were no differences ($P > 0.05$) among the treatments studied at any day of treatment. Feed consumption also increased in all treatments during the 28-d intoxication period, varying from 642-953 $\text{g}\cdot\text{day}^{-1}$ (day 7) to 1429-1586 $\text{g}\cdot\text{day}^{-1}$ (day 28). Pigs from the control group had higher ($P < 0.05$) feed consumption only on day 7, compared with groups receiving 6.1 or 9.0 $\mu\text{g}\cdot\text{g}^{-1}$ of FB_1 . As expected, the FB_1 intake increased ($P < 0.05$) with increasing FB_1 levels in the experimental diets on all days of exposure, varying from 187.9-441.4 $\mu\text{g}\cdot\text{kg}^{-1}$ $\text{bw}\cdot\text{day}^{-1}$ (day 7) to 150.0-470.4 $\mu\text{g}\cdot\text{kg}^{-1}$ $\text{bw}\cdot\text{day}^{-1}$ (day 28). However, FB_1 intake did not vary in pigs from a same FB_1 -treated group during 28 days, except for pigs receiving 6.1 $\mu\text{g}\cdot\text{g}^{-1}$ on day 28, which was lower ($P < 0.05$) than the FB_1 intake on day 14.

Table 1

Performance characteristics of the analytical methods used for fumonisin B₁ analysis of samples of pig plasma, urine and feces.

Type of sample	LOD ($\mu\text{g.L}^{-1}$) ^a	LOQ ($\mu\text{g.L}^{-1}$) ^a	Calibration curve ($\mu\text{g.L}^{-1}$) ^a	r^2	Spiking level ($\mu\text{g.L}^{-1}$) ^a	Recovery ^b (%)	Intra-DP ^c (%)	Inter-DP ^d (%)
Plasma	0.014	0.04	0.04-0.40	0.9937	0.04	70	2.0	3.0
					0.10	77	2.0	1.7
					0.20	72	3.0	2.0
					0.40	78	2.0	1.0
Urine	0.040	0.10	0.1- 50.00	0.9966	0.10	100	2.5	2.0
					25.00	106	1.3	4.0
					50.00	99	1.6	2.0
Feces	0.012	0.04	0.04-2.60	0.9984	0.04	77	5.0	3.0
					0.40	79	4.0	1.5
					2.60	76	2.0	3.0

LOD: Limit of detection; LOQ: Limit of quantification.

^a For feces, all values are expressed as $\mu\text{g}\cdot\text{g}^{-1}$.

^b Mean ($n = 5$).

^c Intra-DP: Intra-day precision, assessed by five replicates analyzed in the same day ($n = 5$).

^d Inter-DP: Inter-day precision, assessed by five replicates analyzed in three different days ($n = 15$).

Table 2

Body weight, feed consumption and fumonisin B₁ (FB₁) intake of pigs fed control or FB₁-contaminated diets over 28 days.

Parameter / Treatment	Days of treatment			
	7	14	21	28
<i>Body weight (kg)¹:</i>				
0 (control)	13.3 ± 1.8 ^{A,c}	17.0 ± 3.1 ^{A,bc}	21.8 ± 4.6 ^{A,b}	28.4 ± 5.0 ^{A,a}
3.1 $\mu\text{g FB}_1\cdot\text{g}^{-1}$	13.4 ± 2.5 ^{A,c}	17.4 ± 3.3 ^{A,bc}	23.6 ± 4.7 ^{A,ab}	30.2 ± 6.7 ^{A,a}
6.1 $\mu\text{g FB}_1\cdot\text{g}^{-1}$	14.0 ± 1.7 ^{A,d}	19.6 ± 2.3 ^{A,c}	25.3 ± 2.5 ^{A,b}	32.0 ± 3.2 ^{A,a}
9.0 $\mu\text{g FB}_1\cdot\text{g}^{-1}$	13.4 ± 2.3 ^{A,c}	17.6 ± 2.5 ^{A,c}	23.6 ± 3.8 ^{A,b}	30.7 ± 3.5 ^{A,a}
<i>Feed consumption (g.day⁻¹)²:</i>				
0 (control)	953 ± 246 ^{A,b}	930 ± 203 ^{AB,b}	1291 ± 107 ^{A,a}	1429 ± 77 ^{A,a}
3.1 $\mu\text{g FB}_1\cdot\text{g}^{-1}$	791 ± 16 ^{AB,b}	815 ± 92 ^{B,b}	1315 ± 92 ^{A,ab}	1435 ± 265 ^{A,a}
6.1 $\mu\text{g FB}_1\cdot\text{g}^{-1}$	699 ± 14 ^{B,c}	1114 ± 161 ^{A,b}	1360 ± 59 ^{A,a}	1479 ± 210 ^{A,a}
9.0 $\mu\text{g FB}_1\cdot\text{g}^{-1}$	642 ± 34 ^{B,d}	874 ± 31 ^{B,c}	1249 ± 85 ^{A,b}	1586 ± 95 ^{A,a}
<i>FB₁ intake ($\mu\text{g}\cdot\text{kg}^{-1}\text{ bw}\cdot\text{day}^{-1}$)²:</i>				
0 (control)	0	0	0	0
3.1 $\mu\text{g FB}_1\cdot\text{g}^{-1}$	187.9 ± 40.4 ^{C,a}	147.1 ± 20.3 ^{C,a}	176.2 ± 31.7 ^{C,a}	150.0 ± 29.5 ^{C,a}
6.1 $\mu\text{g FB}_1\cdot\text{g}^{-1}$	308.6 ± 35.7 ^{B,ab}	350.3 ± 52.0 ^{B,a}	331.2 ± 28.7 ^{B,ab}	283.4 ± 37.0 ^{B,b}

9.0 $\mu\text{g FB}_1\cdot\text{g}^{-1}$ 441.4 \pm 71.5 ^{A,a} 457.4 \pm 77.1 ^{A,a} 486.2 \pm 74.6 ^{A,a} 470.4 \pm 51.1 ^{A,a}

¹ Values are expressed as mean \pm SD for 6 animals in each treatment.

² Values are expressed as mean \pm SD for 2 groups of 3 animals in each treatment.

^{A-C} In the same column, means followed by different capital superscript letters are significantly different ($P < 0.05$).

^{a-d} In the same row, means followed by different lower case superscript letters are significantly different ($P < 0.05$).

3.3. Residues of FB_1 in samples from experimental pigs

FB_1 was not detected in plasma, urine, feces or hair samples from pigs fed control diets (no FB_1 inclusion). The concentrations of FB_1 in samples of plasma, urine and feces from experimental pigs are presented in Table 3. Plasma levels ranged from 0.15-0.18 $\mu\text{g}\cdot\text{L}^{-1}$, 0.26-0.36 $\mu\text{g}\cdot\text{L}^{-1}$ and 0.42-1.08 $\mu\text{g}\cdot\text{L}^{-1}$ for groups fed FB_1 -contaminated diets containing 3.1 $\mu\text{g}\cdot\text{g}^{-1}$, 6.1 $\mu\text{g}\cdot\text{g}^{-1}$ and 9.0 $\mu\text{g}\cdot\text{g}^{-1}$, respectively. Compared with the other pigs fed FB_1 -contaminated diets, FB_1 levels in plasma were higher ($P < 0.05$) in samples from pigs receiving the highest FB_1 level (9.0 $\mu\text{g}\cdot\text{g}^{-1}$) at all days of exposure. Plasma concentrations of FB_1 in pigs from the same group remained constant ($P > 0.05$) throughout the experiment, except for animals fed 9.0 $\mu\text{g}\cdot\text{g}^{-1}$, where values decreased ($P < 0.05$) from day 7 (1.08 \pm 0.52 $\mu\text{g}\cdot\text{L}^{-1}$) to day 28 (0.42 \pm 0.10 $\mu\text{g}\cdot\text{L}^{-1}$).

A high variability in the urinary levels of FB_1 was observed among pigs fed FB_1 -contaminated diets, with no differences ($P > 0.05$) between FB_1 -treated groups at any day of exposure (Table 3). However, similar to levels found in plasma, pigs fed 9.0 $\mu\text{g}\cdot\text{g}^{-1}$ had lower ($P < 0.05$) concentrations of FB_1 in urine on day 28 (18.88 \pm 4.41 $\mu\text{g}\cdot\text{L}^{-1}$), when compared with day 7 (75.01 \pm 55.20 $\mu\text{g}\cdot\text{L}^{-1}$). FB_1 levels in feces varied from 1.87-3.49 $\mu\text{g}\cdot\text{g}^{-1}$, 5.20-10.62 $\mu\text{g}\cdot\text{g}^{-1}$ and 8.15-13.89 $\mu\text{g}\cdot\text{g}^{-1}$ for groups fed 3.1 $\mu\text{g}\cdot\text{g}^{-1}$, 6.1 $\mu\text{g}\cdot\text{g}^{-1}$ and 9.0 $\mu\text{g}\cdot\text{g}^{-1}$ of FB_1 ,

respectively. The concentrations of FB₁ were lower ($P < 0.05$) in feces from pigs fed 3.1 $\mu\text{g}\cdot\text{g}^{-1}$ of FB₁, compared with the other FB₁-treatment groups on days 14 and 21 of exposure, and with pigs fed 9.0 $\mu\text{g}\cdot\text{g}^{-1}$ FB₁ on day 28. No variations in the FB₁ levels in feces were observed over time, except for pigs fed 6.1 $\mu\text{g}\cdot\text{g}^{-1}$, which had higher ($P < 0.05$) levels than the other groups on day 21.

Table 3

Fumonisin B₁ (FB₁) levels in plasma, urine and feces from pigs fed FB₁-contaminated diets during 28 days¹.

Sample / Treatment	Days of treatment			
	7	14	21	28
<i>Plasma ($\mu\text{g}\cdot\text{L}^{-1}$):</i>				
3.1 μg FB ₁ ·g ⁻¹	0.16 ± 0.05 ^{B,a}	0.15 ± 0.05 ^{B,a}	0.18 ± 0.04 ^{B,a}	0.16 ± 0.04 ^{B,a}
6.1 μg FB ₁ ·g ⁻¹	0.35 ± 0.15 ^{B,a}	0.36 ± 0.17 ^{B,a}	0.32 ± 0.11 ^{B,a}	0.26 ± 0.06 ^{B,a}
9.0 μg FB ₁ ·g ⁻¹	1.08 ± 0.52 ^{A,a}	0.80 ± 0.32 ^{A,ab}	0.77 ± 0.27 ^{A,ab}	0.42 ± 0.10 ^{A,b}
<i>Urine ($\mu\text{g}\cdot\text{L}^{-1}$):</i>				
3.1 μg FB ₁ ·g ⁻¹	28.53 ± 17.95 ^{A,a}	17.76 ± 20.10 ^{A,a}	31.40 ± 30.60 ^{A,a}	16.09 ± 21.94 ^{A,a}
6.1 μg FB ₁ ·g ⁻¹	47.34 ± 26.40 ^{A,a}	22.65 ± 17.40 ^{A,a}	23.07 ± 11.47 ^{A,a}	24.08 ± 25.96 ^{A,a}
9.0 μg FB ₁ ·g ⁻¹	75.01 ± 55.20 ^{A,a}	37.45 ± 13.26 ^{A,ab}	55.24 ± 15.79 ^{A,ab}	18.88 ± 4.41 ^{A,b}
<i>Feces ($\mu\text{g}\cdot\text{g}^{-1}$):</i>				
3.1 μg FB ₁ ·g ⁻¹	2.27 ± 1.64 ^{A,a}	1.87 ± 1.13 ^{B,a}	3.49 ± 2.28 ^{B,a}	2.25 ± 1.88 ^{B,a}
6.1 μg FB ₁ ·g ⁻¹	5.20 ± 1.64 ^{A,b}	6.83 ± 2.28 ^{A,b}	10.62 ± 2.38 ^{A,a}	5.84 ± 2.94 ^{AB,b}
9.0 μg FB ₁ ·g ⁻¹	8.15 ± 6.80 ^{A,a}	9.66 ± 3.89 ^{A,a}	13.89 ± 4.89 ^{A,a}	13.48 ± 7.59 ^{A,a}

¹ Values are expressed as mean ± SD for 6 animals in each treatment.

^{A-C} In the same column, means followed by different capital superscript letters differ significantly ($P < 0.05$).

^{a-b} In the same row, means followed by different lower case superscript letters differ significantly ($P < 0.05$).

Results of FB₁ levels found in pooled hair samples ($n = 3$) from pigs fed FB₁-contaminated diets over 28 days are presented in Figure 1. The mean FB₁ concentrations were $2.08 \pm 1.23 \text{ ng.g}^{-1}$, $8.09 \pm 9.93 \text{ ng.g}^{-1}$ and $7.52 \pm 6.60 \text{ ng.g}^{-1}$ for groups fed $3.1 \text{ }\mu\text{g.g}^{-1}$, $6.1 \text{ }\mu\text{g.g}^{-1}$ and $9.0 \text{ }\mu\text{g.g}^{-1}$ of FB₁, respectively, although no significant differences were found among means ($P > 0.05$). This is the first report on FB₁ levels in the hair of pigs fed fumonisin-contaminated diets. Figure 2A-D presents the chromatograms (quantification transitions) of FB₁ in samples of plasma (2A) and urine (2B) collected from a pig fed $9.0 \text{ }\mu\text{g FB}_1.\text{g}^{-1}$ at the 14th day of exposure, fecal sample (2C) collected from a pig fed $3.1 \text{ }\mu\text{g FB}_1.\text{g}^{-1}$ at the 7th day of exposure, and pooled hair sample (2D) containing 1.78 ng.g^{-1} , collected from 3 pigs fed $9.0 \text{ }\mu\text{g FB}_1.\text{g}^{-1}$ at the 21st day of exposure. Thus, taken together, the FB₁ results in samples from experimental pigs indicate that mean FB₁ levels in urine were about 10 and 100 times higher than the mean levels found in hair and plasma, respectively.

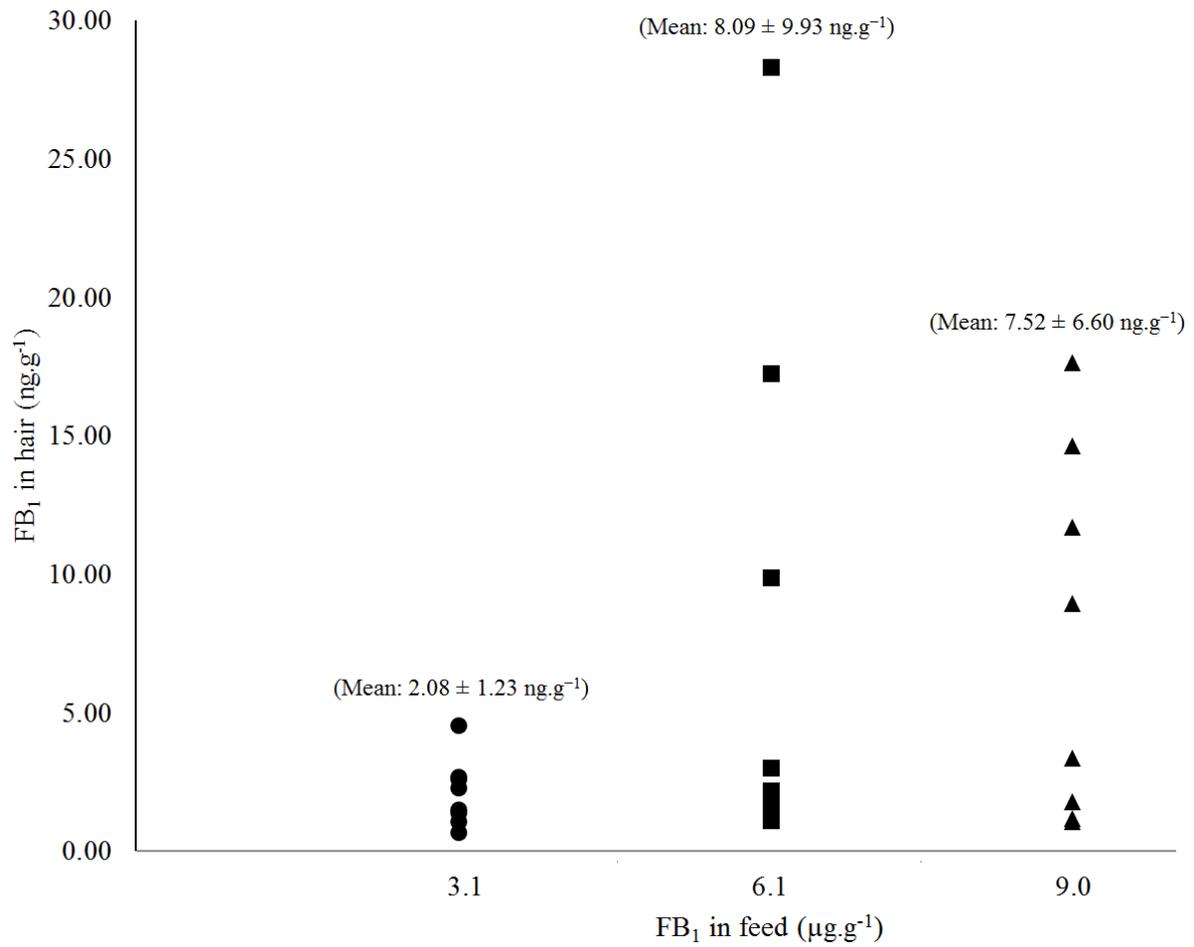


Figure 1. Fumonisin B₁ (FB₁) levels in pooled hair samples ($n = 3$) from pigs fed FB₁-contaminated diets over 28 days. No significant differences ($P < 0.05$) were found among means from treatments.

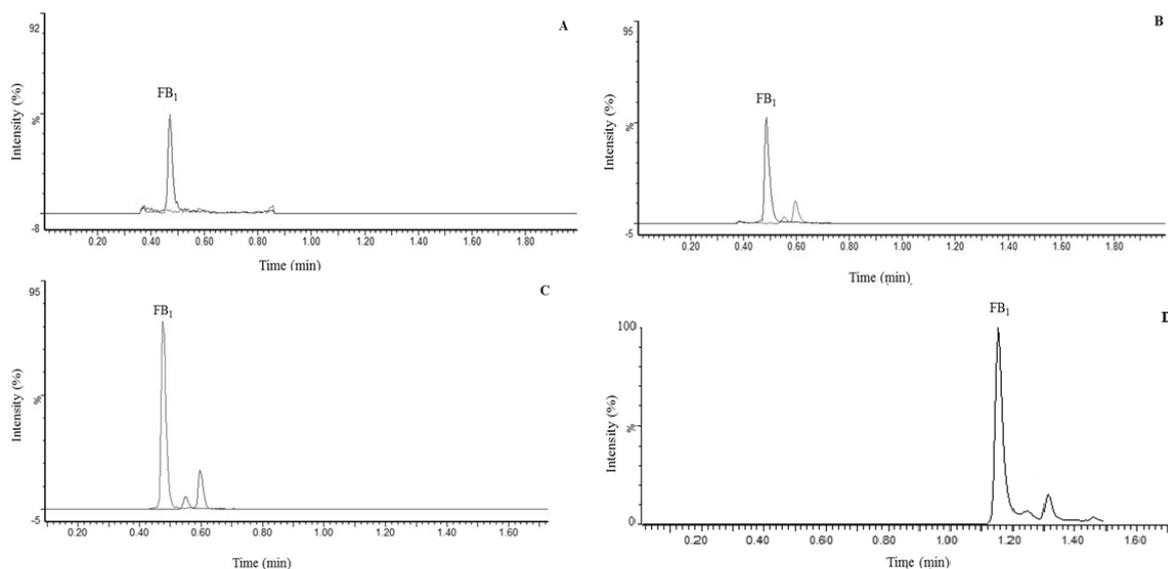


Figure 2. Chromatograms (quantification transitions) of fumonisin B₁ (FB₁) in: A) Plasma sample containing 1.02 $\mu\text{g}\cdot\text{L}^{-1}$, collected from a pig fed 9.0 $\mu\text{g}\cdot\text{g}^{-1}$ at the 14th day of exposure; B) Urine sample containing 27.6 $\mu\text{g}\cdot\text{L}^{-1}$, collected from a pig fed 9.0 $\mu\text{g}\cdot\text{g}^{-1}$ at the 14th day of exposure; C) Fecal sample containing 3.94 $\mu\text{g}\cdot\text{g}^{-1}$, collected from a pig fed 3.1 $\mu\text{g}\cdot\text{g}^{-1}$ at the 7th day of exposure. D) Pooled hair sample containing 1.78 $\text{ng}\cdot\text{g}^{-1}$, collected from 3 pigs fed 9.0 $\mu\text{g}\cdot\text{g}^{-1}$ at the 21st day of exposure.

3.4. Correlations between FB₁ intake and residual FB₁ in plasma, urine and hair

The data from plasma, urine and pooled hair samples revealed a high correlation ($r = 0.739$; $P < 0.001$; $N = 72$) between the FB₁ concentrations in plasma and the FB₁ intake over the 28 days of experiment, as shown in Figure 3A. The correlation between urinary FB₁ levels and FB₁ intake (Figure 3B) was also significant ($r = 0.329$; $P < 0.01$; $N = 72$) although lower than the values obtained for plasma. Moreover, a modest though significant correlation ($r = 0.509$; $P = 0.02$; $N = 24$) was found between the FB₁ concentrations in pooled hair samples and the FB₁ intake by pigs (Figure 3C).

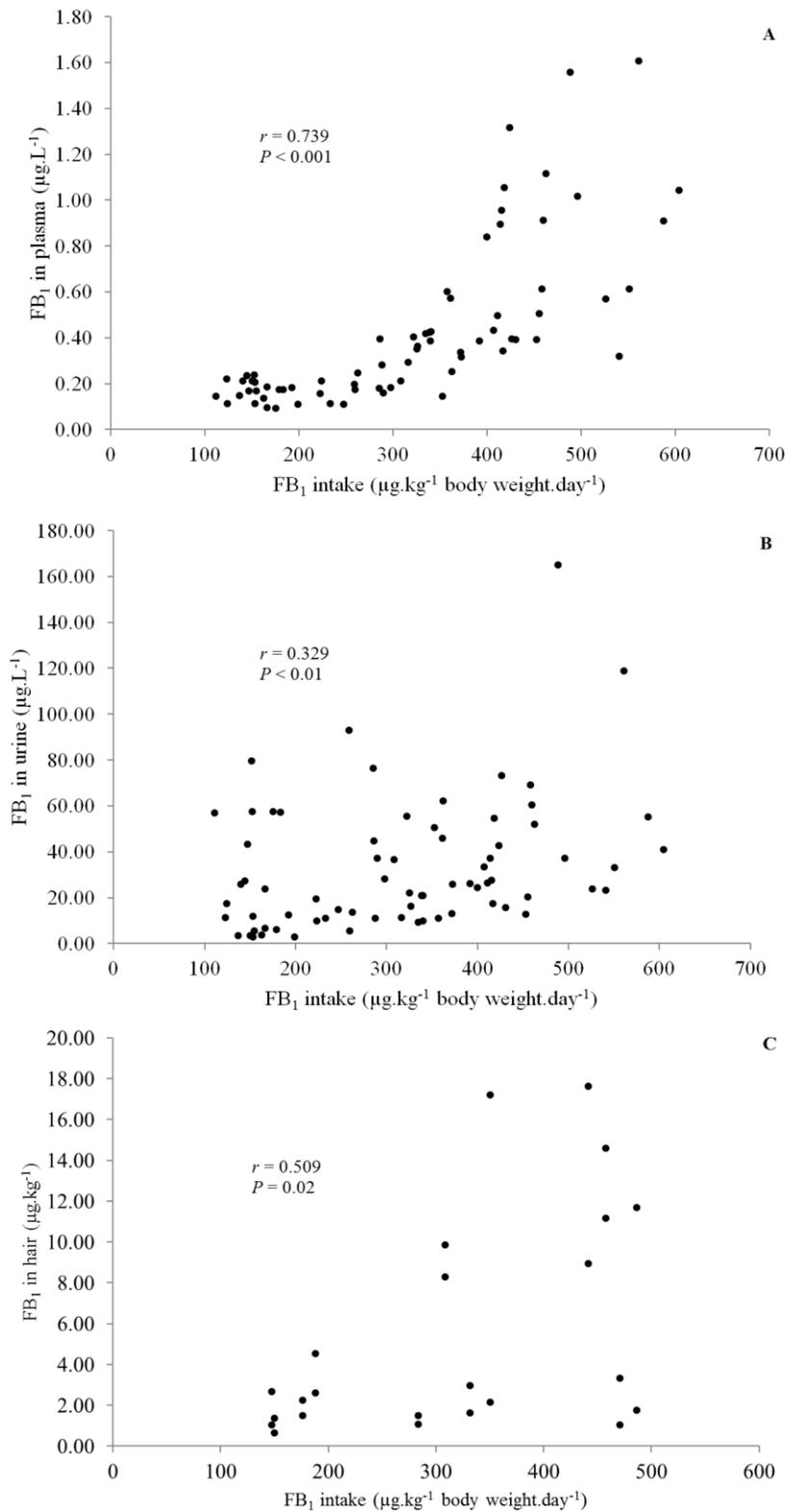


Figure 3. The relationships between fumonisin B₁ (FB₁) daily intake and the respective levels of FB₁ in plasma (A), urine (B) or pooled ($n = 3$) hair (C) samples from pigs fed FB₁-contaminated diets over 28 days (Plasma or urine, $N = 72$; Hair, $N = 24$).

The correlation coefficients between FB₁ intake and residual FB₁ in samples of plasma and urine after 7, 14, 21 and 28 days of exposure are presented in Table 4. Higher *r* values (*r* = 0.808-0.885; *P* < 0.001; *N* = 18) were observed for plasma on all days of treatment. Correlations between FB₁ intake and urinary FB₁ levels were significant only on days 7 (*r* = 0.561; *P* < 0.02; *N* = 18) and 14 (*r* = 0.572; *P* < 0.02; *N* = 18) of the exposure period.

Table 4

Correlation coefficients (*r*) between fumonisin B₁ (FB₁) intake ($\mu\text{g}\cdot\text{kg}^{-1}$ body weight $\cdot\text{day}^{-1}$) and the respective levels ($\mu\text{g}\cdot\text{L}^{-1}$) in plasma or urine from pigs fed FB₁-contaminated diets during 28 days.

Days of treatment	Plasma ¹		Urine ¹	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
7	0.885	< 0.001	0.561	0.02
14	0.817	< 0.001	0.572	0.02
21	0.808	< 0.001	0.249	0.40
28	0.859	< 0.001	0.026	0.90

¹ *N* = 18 for each day of treatment.

4. Discussion

4.1. Method performance

In the present study, samples of plasma, urine and feces from pigs fed FB₁-contaminated diets were analyzed by simply diluting the samples to reduce matrix effects and subsequently injecting into the LC-MS/MS system. The recoveries rates and coefficient of variation values obtained at different spiking levels of FB₁ in blank samples of plasma urine

and feces were satisfactory according to the European Commission Regulation No. 401/2006 (European Community 2006). Thus the analytical method was adequate to detect and quantify FB₁ residues in the matrices evaluated from pigs fed diets containing relatively low levels of FB₁. The performance characteristics as described in this study for urine were similar to those reported by Gambacorta et al. (2013) for an analytical method used to evaluate urinary FB₁ levels in pigs after oral ingestion of FB₁ and other mycotoxins. Our results on the method's performance for analysis of plasma are also in agreement with recovery rates and coefficient of variation values of an analytical method described by Devreese et al. (2012) for FB₁ and other mycotoxins in pig plasma. For feces, recoveries for the analytical method used were comparable with results obtained by Fodor et al. (2008). The analytical method for FB₁ in hair was previously validated using human samples and the results were described by Bordin et al. (2014). The mean recovery was 86.6%, with a relative standard deviation of 12.4% for FB₁ levels of 20 and 40 ng g⁻¹.

4.2. Growth performance and FB₁ intake by experimental pigs

Overall, the FB₁ levels in diets used in the present study (3.1-9.0 µg.g⁻¹) did not significantly affect the body weight gain or feed consumption of pigs over 28 days of exposure. These results are in agreement with previous data available in the literature, reporting that levels up to 11.8 µg.g⁻¹ of FB₁ did not affect the performance of pigs (Zomborszky et al. 2002; Halloy et al 2005; Dilkin et al. 2010; Grenier et al. 2011; Burel et al. 2013). Adverse effects in pigs were observed only after oral administration of higher FB₁ levels (>30 µg.g⁻¹) (Ahangarkani et al. 2014). The FB₁ intake, calculated on the basis of FB₁ levels in the diet, feed consumption and body weight, increased with increasing levels of FB₁ in feed, but overall did not vary in pigs from the same FB₁-treated group over 28 days. These results are in agreement with data indicating that body weight and feed consumption were not

affected by the dietary FB₁ levels used over the 28 days. However ingestion of such doses of FB₁ induces deleterious effect in pigs (Devriendt et al. 2009; Grenier et al. 2012; Loiseau et al 2015).

4.3. FB₁ residues in samples from experimental pigs

The mean FB₁ levels found in plasma from all exposed pigs (0.15-1.08 µg.L⁻¹) are in agreement with previous studies indicating that FB₁ bioavailability in pigs is very low, less than 3.5% (Martinez-Larrañaga et al. 1999). Explanations for the low absorption rate of FB₁ are difficult to access at this time. Possible mechanisms could involve strong association between FB₁ and the intestinal contents and/or intense enterohepatic circulation (Prelusky et al. 1994; Dilkin et al. 2010). The plasma levels described were also coherent with data reported by Prelusky et al. (1994) after orally dosing pigs with 500 µg.kg⁻¹ body weight. Using a LC method with fluorescence detection, Dilkin et al. (2010) observed mean FB₁ levels up to 282 µg.L⁻¹ in plasma from pigs given a single oral dose of 5,000 µg.kg⁻¹ body weight, which is much higher than the maximum FB₁ intake reported in the present study (486.2 µg.kg⁻¹ body weight.day⁻¹). Also using a LC method with fluorescence detection, Cai et al. (2007) did not find detectable levels of FB₁ in serum of F344 rats receiving 1,000 or 2,500 µg.kg⁻¹ body weight.day⁻¹ for 5 weeks. Possible explanations for this result is that serum levels were below the detection limit of the method, or most of the absorbed FB₁ bound to serum components such as lipids or serum proteins, hence making the free toxin undetectable. In our study, the high sensitive LC-MS/MS method used demonstrated that plasma concentrations of FB₁ in pigs from groups receiving 3.1 or 6.1 µg.g⁻¹ remained constant from days 7 to 28. However, the fact that pigs fed 9.0 µg.g⁻¹ had decreased FB₁ levels in plasma during the trial suggests an increased enterohepatic circulation in those pigs,

similarly to previous experiments with pigs exposed to high oral doses of FB₁ (Prelusky et al. 1994; Dilkin et al. 2010).

The high individual variability in the urinary FB₁ levels of pigs fed FB₁-contaminated diets was in agreement with data from previous reports (Dilkin et al. 2003; Dilkin et al. 2010). In our study, the mean FB₁ levels in urine were about 100 times higher than the mean levels found in plasma. However, similar to plasma results, the overall mean FB₁ levels found in urine (16.09-75.01 $\mu\text{g}\cdot\text{L}^{-1}$) corroborates the low FB₁ bioavailability in pigs. Gambacorta et al. (2013) observed that recovery of urinary FB₁ in pigs fed 3.71-150.19 $\mu\text{g}\cdot\text{kg}^{-1}$ body weight $\cdot\text{day}^{-1}$ was only 2.6% of the ingested toxin after 24 hours. This value is closer than the percentage (1.5%) described by Fodor et al. (2008) on the total FB₁ excreted in urine after 10 days of exposure.

The overall mean levels of FB₁ in feces (1.87-13.89 $\mu\text{g}\cdot\text{g}^{-1}$) were similar to those reported by Dilkin et al. (2003), who observed mean concentrations of 8.7 ± 1.6 $\mu\text{g}\cdot\text{g}^{-1}$ after 24 h of exposure of pigs with 10 $\mu\text{g}\cdot\text{g}^{-1}$ of feed. The FB₁ levels in feces from pigs receiving 9.0 $\mu\text{g}\cdot\text{g}^{-1}$ did not differ among days of treatment, although the values increased during the treatment period, which is coherent with the decreased FB₁ levels found in plasma and urine from this treatment group. The high concentrations of FB₁ found in feces of all treatment groups in the present study are also in agreement with data described by Prelusky et al. (1994), indicating that more than 90% of the ingested toxin can be recovered in feces of pigs.

The FB₁ levels found in pooled hair samples from pigs (2.08-8.09 $\text{ng}\cdot\text{g}^{-1}$) confirms the suitability of this matrix for assessing the individual exposure to FB₁-contaminated diets in pigs. Moreover, the residual FB₁ levels in pooled hair were about 10 times higher or lower than the concentration range observed in plasma or urine, respectively. There is no previous study on the occurrence of FB₁ in pig hair, only one report with nonhuman primates and rats (Sewram et al. 2001) and two reports on the incidence of the toxin in human hair in South

Africa (Sewram et al., 2003) and in Brazil (Bordin et al. 2015). The reported levels of residual FB₁ in hair from primates and rats were much higher than the concentrations described in hair from pigs in the present experiment, possibly because the FB₁ exposure was also higher in those studies. In hair from primates exposed to nearly 30 µg or 800 µg of total fumonisins.kg⁻¹ body weight.day⁻¹, FB₁ was detected at concentrations of 33.77 ng.g⁻¹ and 65.93 ng.g⁻¹, respectively. In rats receiving a single oral dose of FB₁ (10,000 µg.g⁻¹ body weight) or dietary FB₁ (50 µg.g⁻¹), FB₁ was found in hair at concentrations of 34.50 ng.g⁻¹ and 42.20 ng.g⁻¹, respectively (Sewram et al. 2001). In human hair, FB₁ was found at concentrations of 27.0-33.0 ng.g⁻¹ (Sewram et al. 2003) or 21.3 ng.g⁻¹ (Bordin et al. 2015), which are also higher than the levels described in the present study. The estimated daily intakes of FB₁ by subjects in the studies by Sewram et al. (2003) and Bordin et al. (2015) were lower than those reported in our experiment 13.8 µg.kg⁻¹ bw day⁻¹ and 0.16 µg.kg⁻¹ bw day⁻¹, respectively, hence indicating that the process of fumonisin deposition in the hair varies markedly between different species. In rats, Sewram et al. (2001) observed that hair grew again after 28 days of a first sampling, indicating that this period was sufficient for accumulation of detectable levels of FB₁ in the newly grown hair. There are no previous studies about the hair growth rate in pigs exposed to FB₁, or accumulation of the toxin in this matrix. However, recent studies have been proposed pig hair for veterinary drug incorporation analyses to monitor the overdosing or illegal use of drugs in pigs (Huang et al., 2016; Decheng et al., 2016), thus confirming the increased interest in using pig hair analyses for biomonitoring past exposures of toxic compounds.

4.4. Correlations between FB₁ intake and residual FB₁ in experimental pigs

The correlation coefficients between FB₁ intake and residual FB₁ indicated that plasma is the better matrix to assess the individual dietary exposure to the toxin at any day over 28

days of exposure. No previous study on the correlation between FB₁ intake and respective levels of FB₁ in plasma was found in the literature. Our results indicate that plasma concentrations of FB₁ in pigs remain relatively constant during continuous exposure to low levels of FB₁ in the diet. However, urinary FB₁ is a valuable biomarker only during the initial 14 days of exposure, which is in agreement with data reported previously (Gambacorta et al. 2013). The poor correlations found between fumonisin intake and FB₁ excreted in urine at 21 or 28 days are consistent with the high variability in the urinary levels of FB₁ observed among pigs fed FB₁-contaminated diets at those days of exposure (Table 3). These results can be associated with decreasing FB₁ bioavailability in pigs during time, although variations in hydrolysis capacity may also be involved since the FB₁ amount excreted in pig urine comprises nearly 65% of the original compound ingested after 10 days of exposure, with 16% excreted as totally hydrolysed (aminopentol) and 24% as a partially hydrolysed form (Fodor et al., 2008).

There are no previous studies on the correlation between FB₁ intake by animal models and residual levels in the hair. In the present study, the fact that FB₁ concentrations in pooled hair samples correlated with the FB₁ intake by pigs indicate that this matrix may also be useful for estimation of exposure to FB₁. Moreover, results of this trial are consistent with data reported by Bordin et al. (2015), who found that FB₁ levels in human hair correlated with FB₁ probable daily intake values from volunteers in Brazil. Recently, Mupunga et al. (2016) hypothesized that hair can be a potential matrix for biomarkers of long-term human exposure to aflatoxins. However, hair analysis is subject to certain limitations, such as the occurrence of exogenous contamination deposits of sebum, sweat, polluted air residues or residues of cosmetic, which could interfere in the analysis of contaminants (Rodrigues et al., 2008). Therefore further studies are needed to provide physiologically based toxicokinetics of residual FB₁ in the pig hair.

In conclusion, results of this trial demonstrate that both plasma and urinary FB₁ are good biomarkers of early exposure of pigs to low dietary levels of FB₁, although plasma levels are recommended under conditions of prolonged exposure (> 14 days). The possibility to evaluate hair as a biomarker to assess the dietary exposure of pigs to fumonisins was also established.

Authors' contributions

PS, IPO and CO designed the study and analyzed the data. PS, PP and AMC conducted the experiment with pigs. PS, AJ and TP carried out the FB₁ analyses. FT optimized the LC-MS/MS analytical parameters. PS and MG drafted the manuscript. GR supplied the FB₁ culture material, supervised FB₁ analyses in hair samples and made intellectual contributions for the manuscript. CO and IPO performed a final, critical review of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

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