



## Note

### Occurrence of fumonisins in feed for swine and horses

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#### ABSTRACT

**Background:** Fumonisin B1 (FB1), fumonisin B2 (FB2), and overall mycotoxins feed contamination may cause several effects on crops production and animal health. The contamination occurred predominantly in corn and corn-based foods and feeds.

**Aims:** This survey intends to provide the occurrence of fumonisins in swine and equine mixed feeds in Portugal, making an overview from 2007 to 2010.

**Methods:** A total of 363 samples were analyzed, 258 from swine feed and 105 from horse feed with HPLC method. The detection limit was 50 µg/kg for FB1 and 100 µg/kg for FB2.

**Results:** The overall results were 13% of FB1 occurrence from 2007 to 2010. FB1 was detected in about 17.0% of swine feed samples, being more frequent in 2010 (32.9%). In this year (2010) levels ranged between 66.7 and 3815.5 µg/kg.

FB2 occurred only in 2010 in swine feed (6 samples, ranging between 104.0 to 467.2 µg/kg) and in horse feed (1 sample).

**Conclusions:** This represents an increase in occurrence through the analyzed years, but this may not be a threat to animal health, once the values were below the recommended guidance values from European Commission.

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### Incidencia de fumonisinas en piensos para cerdos y caballos

#### RESUMEN

**Antecedentes:** La contaminación por fumonisina B1 (FB1) y B2 (FB2) y por el conjunto de micotoxinas en alimentos y piensos puede causar efectos diversos sobre los productos agrícolas y la salud de los animales. La contaminación afectó de modo predominante al maíz y a los alimentos y piensos derivados.

**Objetivos:** En este estudio, efectuada en Portugal, se examinó la incidencia de fumonisinas en muestras de pienso mixto para cerdos y para caballos. El período de la revisión abarcó de 2007 a 2010.

**Métodos:** Mediante cromatografía líquida de alta resolución (HPLC) se analizaron 363 muestras, 258 de pienso porcino y 105 de pienso equino. El límite de detección fue de 50 µg/kg para FB1 y de 100 µg/kg para FB2.

**Resultados:** Los resultados globales fueron una incidencia de contaminación por fumonisina B1 (FB1) del 13% desde 2007 a 2010. Se detectó FB1 en alrededor del 17% de las muestras de pienso porcino, siendo más frecuente en 2010 (32,9%). En dicho año, los niveles variaron entre 66,7 y 3.815,5 µg/kg.

En 2010 sólo se detectó contaminación por fumonisina B2 en muestras de pienso para cerdo (seis muestras, con una variación de 104,0 a 467,2 µg/kg) y en muestras de pienso para caballos (una muestra).

**Conclusiones:** Aunque los resultados de este estudio indican un aumento de la incidencia durante los años examinados, no constituye un riesgo para la salud de los animales, ya que los valores no superan los recomendados por las directrices de la Comisión Europea.

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The group of fumonisins (FBs), discovered in the late eighties and belonging to the *Fusarium* toxins, causes many losses in agricultural crops, mainly in maize.<sup>10,17</sup> Once maize is one of the most used crops in the world, even as a feed compound, economical losses can be enormous. The toxic effects may increase with both multi-exposure and synergic effects, which can be observed when feeds are contaminated with several mycotoxins.<sup>14</sup>

Chemical structures of FBs are similar to those of sphingosine and sphinganine, which are sphingoid bases that inhibit the ceramid synthase, resulting in a disruption in the lipid metabolism.<sup>17</sup> This is the reason why accumulation of biomarkers as free sphingoid bases in the serum and urine, indicate fumonisins exposure.<sup>11</sup>

Toxicity of FBs for humans has been associated with esophageal cancer, with an increasing incidence mainly in regions where maize is the base of the diet of populations.<sup>10</sup> In animals FBs can cause deep lesions in the liver, gastrointestinal tract, nervous system and lungs.<sup>18</sup> Many authors refer that horses are the most sensitive species to this mycotoxin.<sup>9,10,14</sup> It can affect the central nervous system with brain damage, causing a disease called equine leukoencephalomalacia (ELEM).<sup>10,17</sup> The signs begin with depression, lack of appetite after a period of eating contaminated feed and lethargy.<sup>15</sup> Clinical effects such as ataxia, apathy hypersensitivity, impaired locomotor function, necrosis of cerebral white matter and lesions in the cerebral cortex,<sup>9</sup> have also been reported.

In swine, FBs can cause porcine pulmonary oedema (PPE),<sup>5,8,14</sup> including symptoms such as dyspnea, cyanosis, and even death.<sup>9</sup> Reporting to some surveys,<sup>9</sup> the swine immune system response has been incoherent. Other symptoms are related to heart and respiratory dysfunctions.

The purpose of this work was to give an overview of the occurrence of FBs in feed for swine and horses in Portugal, and to compare the results with data from other years.

A total of 468 feed samples (258 for swine and 105 for horses) were collected during 4 years (2007–2010) from compound feed factories from all regions of Portugal, in a random mode and under a previously established plan, according to the Commission Regulation No 152/2009.<sup>4</sup> All samples were collected from factories, with HACCP plan adequately implemented, fulfilling Regulation (EC) No 1831/2003<sup>3</sup> laying down requirements for feed hygiene.

All reagents were of analytical grade, and solvents used for chromatography were of analytical-grade purity, provided by VWR (Germany). Standard of FBs was obtained from Sigma Chemicals Company (Spain).

The methods performed to quantify FBs is described in the international standard EN 14352 (2004),<sup>1</sup> using High Performance Liquid Chromatography and purification with immunoaffinity columns. The technique may be described, briefly, as following: 50 ml of extraction solvent (acetonitrile/methanol/water) was added to 20 g of milled sample and shaken for 20 min. It was centrifuged (2500 × g for 10 min) and the supernatant was fil-

tered. 50 ml of solvent was added to the remaining solid material, centrifuged and filtered again. The two extract filtrates were mixed. Forty milliliters of PBS (phosphate buffered saline) was added to 10 ml of the extract and filtered. Ten milliliters of the collected filtrate was cleaned up through immunoaffinity column (VICAM-FumoniTest™ WB, 1060). The toxins were eluted with 1.5 ml methanol. The eluate was evaporated to dryness and the dried sample was redissolved in 200 µl of acetonitrile–water solution before HPLC injection. An aliquot was derivatized with 50 µl O-phthalaldehyde-2-mercaptoethanol (Sigma) and 20 µl HPLC injections were made within 3 min. The analysis was done by a reverse phase using a Hitachi data module with fluorescence detection (excitation and emission wavelengths were 335 and 440 nm, respectively). Mobile phase composition was methanol–phosphate buffer (77 + 23, v/v, pH 3.35). The detection limit was 50 µg/kg and 100 µg/kg for FB1 and FB2, respectively.

The results of FBs are displayed in Table 1. FB1 was detected in 45 samples out of the 258 samples of swine feed. This corresponds to approximately 17.44% of the occurrence during the monitored period. The major occurrence (32.93%) and concentrations (maximum value was 3815.5 µg/kg) were registered in 2010. FB2 occurred only in 2010 in swine and horse feed, in 6 (7.59%) and 1 sample, respectively. The values of FB2 in swine feed ranged from 104.0 to 467.2 µg/kg and 3 of these samples were found to be contaminated with FB1 and FB2, with a lower concentration for FB2. The other 3 positive samples for FB2 were negative for FB1. The concentration value of FB2 presented in horse feed was higher than that of FB1, which is a result of little relevance considering the fact that FB2 is less toxic than FB1.

In a survey conducted in Uruguay, FBs were found in some feed samples in concentrations up to 6342 µg/kg.<sup>13</sup> In 2001, 86% out of 58 Spanish corn-based feed, were contaminated with FBs with levels ranging from 89 to 8757 µg/kg, and four samples had levels of FB1 up to 5000 µg/kg. None of the samples had more than 10,000 µg/kg.<sup>16</sup> Despite comparing different years, the results of our study seem to raise less concern than the results previously reported by other authors.

Griessler et al.<sup>6</sup> investigated feedstuff samples in Southern Europe countries, including Portugal. Occurrence and highest detected levels from 43 feedstuffs samples, 33 were positive (77%) with average concentration 1411 µg/kg for FBs. Out of 43 samples, 15 were finished compounded feed checked for the presence of FBs. The positive samples were 6 (40%) with levels ranging from 100 to 3228 µg/kg (average 622 µg/kg). The same authors reported that in Portugal, out of 10 feed samples, 7 were positive with 99–3093 µg of FBs/kg (average 631 µg/kg). Regarding Portuguese feed samples, these researchers found fumonisins in 7 from 10 samples with 99–3093 µg/kg levels (average 631 µg/kg). These results are similar to those found in the present work, especially during 2010.

In Portugal, Marques et al.<sup>12</sup> presented data from 2000 to 2005, in which the occurrence of FBs was not reported from the 225 swine

**Table 1**  
Trend of fumonisin B1 and B2 occurrence in swine and horse feed in Portugal monitored from 2007 to 2010.

|                     | Swine feed |                      |                      |               | Horse feed |                 |
|---------------------|------------|----------------------|----------------------|---------------|------------|-----------------|
|                     | N+/N       | Positive samples (%) | Average (µg/kg) ± SD | Range (µg/kg) | N+/N       | Maximum (µg/kg) |
| <i>Fumonisin B1</i> |            |                      |                      |               |            |                 |
| 2007                | 1/14       | 7.14                 | –                    | 102.92        | 0/6        | –               |
| 2008                | 7/35       | 20.0                 | 60.77 ± 20.56        | 52.9–110.96   | 0/14       | –               |
| 2009                | 10/127     | 7.87                 | 212.05 ± 133.23      | 53.7–391.4    | 0/54       | –               |
| 2010                | 27/82      | 32.93                | 630.76 ± 885.38      | 66.66–3 815.5 | 1/31       | 79.6            |
| <i>Fumonisin B2</i> |            |                      |                      |               |            |                 |
| 2007–2009           | –          | –                    | –                    | –             | –          | –               |
| 2010                | 6/82       | 7.32                 | 232.85 ± 147.43      | 104.0–467.2   | 1/31       | 138.8           |

N+, positive samples; N, number of samples; SD, standard deviation.

feed analyzed samples. Another survey was conducted in 2005<sup>7</sup> to evaluate the sanitary safety of mixed feed for horses, and the authors verified that fumonisins were not detected in 50 analyzed samples. Comparing our results to these, it was soon observed that in the last years the presence of fumonisins in swine and horse mixed feed was found to be higher. Nevertheless, the difference between the contamination levels of FBs in the studied feed samples and the data reported by other authors may also be attributed to the different origin of ingredients for mixed feed, once cereal infections strongly depend on the environmental conditions. The time of the harvest also can affect results, since fungi contamination is most frequent to happen with high humidity. The interpretation of our results must be done with caution, considering the limited number of samples that were analyzed compared to the dimension of national farms that use compound feeds.

It also should be noticed that although all values of contamination were below the guidance values in 2006/576/EC from European Commission,<sup>2</sup> which is 5000 µg/kg for the analyzed feeds, in 2010 FBs contamination in swine feed samples was notably higher. This observed fact might not be a threat to animal health; however, this trend must be attenuated in order to avoid unsatisfactory results and economic losses. Stronger prevention measures are needed and must be implemented in order to reverse this trend. Full cooperation and commitment of feed operators, including the animal producers is mandatory.

To continue a tight surveillance program of monitoring mycotoxins, this study confirms the importance of FB control in Portuguese feed production. The awareness of animal diseases caused by FBs and adherence to institutional guidelines are important to ensure the minimization of FB- and mycotoxin-related problems in crops and, as a consequence, animal nutrition. As known, mycotoxins are natural contaminants and their presence cannot be eradicated. However, risk management based in epidemiological data with adequate technical procedures on crop storage and systematic monitoring of mycotoxins in feed chain are crucial for an effective FBs control.

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