Occurrence of aflatoxin \(B_1\) in dairy cow’s feed over 10 years in Portugal (1995-2004)

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Summary

In Portugal, there is rather little information about the natural occurrence of aflatoxin in feedstuffs. The aim of this work was to report the results of screening the natural incidence of aflatoxin \(B_1\) (AFB\(_1\)) in samples of cattle feed collected from seven dairy cow’s farms from Portugal distributed in several locations of the country. One thousand and one samples were taken from 1995 to 2004. High performance liquid chromatography (HPLC) was used for separation, identification and quantification of the compound. Detection limit was 1 µg /kg. Aflatoxin \(B_1\) was detected in 374 (37.4%) of the samples. The incidence and mean content of AFB\(_1\) was generally low. Levels of aflatoxin \(B_1\) above the maximum limit established in Portugal (5 µg/kg) for dairy cattle feed samples were observed in 62 samples (6.2%) with levels ranging from 5.1 to 74 µg/kg. Out of those 62 samples, 3.7% had levels between 5.1 to 10 (mean 7.8); 1.8% had a contamination level of 10.1 to 20 (mean 12.0), and 0.7% exceeded 20.1 µg/kg (mean 50.4). On the last two years (2003-04) none of the samples exceeded the maximum permissible level of the toxin.

Key words

Aflatoxin B1, Dairy cattle feed, Occurrence

Presencia de aflatoxina B\(_1\) en piensos para ganado lechero en Portugal durante el periodo 1995-2004

Resumen

En Portugal es muy escasa la información sobre la presencia natural de aflatoxina en piensos para animales. El objetivo de este trabajo es la divulgación del estudio sobre la incidencia natural de aflatoxina \(B_1\) (AFB\(_1\)) en muestras de piensos, recogidas en siete explotaciones de ganaderas de bovinos lecheros distribuidas por diversas regiones de Portugal. Se recogieron y analizaron 1.001 muestras entre el periodo de 1995 a 2004. En la separación, identificación y cuantificación del compuesto, se utilizó la técnica de cromatografía líquida de alta resolución (HPLC). El límite de detección fue de 1 µg/kg. La aflatoxina \(B_1\) fue detectada en 374 (37,4%) de las muestras. La incidencia y el contenido medio de AFB\(_1\) fueron en general, bajos. En 62 muestras (6,2%) se observaron niveles de aflatoxina \(B_1\) por encima del límite máximo permitido en Portugal (5 µg/kg), con concentraciones entre 5,1 y 74 µg/kg. De las 62 muestras, 3,7% presentaron concentraciones entre 5,1 y 10 µg/kg (mediana 7,8); 1,8% tenía una concentración entre 10,1 y 20 µg/kg (mediana 12,0), y 0,7% era superior a 20,1 µg/kg (mediana 50,4). En los últimos 2 años del estudio (2003-04) ninguna muestra excedió el límite máximo permitido para la toxina.

Palabras clave

Aflatoxinas, Ganado lechero, Presencia, Piensos

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Myco toxins in feedstuffs are recognised as a public health problem of considerable importance. Fungal toxins in feed and food have become a major research area since the 1961 discovery of the carcinogenicity of the aflatoxins. Fungal infestation and subsequent myco toxins production can occur during plant growth, maturity, harvesting, processing of grains and is influenced by various factors (temperature, relative humidity, oxygen availability, damaged or broken grain kernels) [1,9].

Aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂, AFM₁, AFM₂), a group of potent carcinogenic and teratogenic compounds, are secondary metabolic products of some Aspergillus spp.: Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius [8,10]. Since the discovery of the aflatoxins, a comprehensive literature on many aspects of their natural occurrence, chemistry and toxicology has been described. AFB₁ may occur in a wide variety of agricultural commodities such as: barley, beans, corn, cottonseed, rice, wheat and many other items like peanuts. Aflatoxins contain a coumarin nucleus fused to a bifuran and either a pentanone (AFB₂, AFB₃) or a six-membered lactone (AFG₁, AFG₂) [2,6]. AFB₁ presents the highest degree of toxicity for animals, followed by aflatoxins M₁ (AFM₁), AFG₁ and AFB₂ (AFG₂) [4].

AFB₁ can increase the incidence of disease and reduce production efficiency. Some of the gross effects of AFB₁ can include: intake reduction or feed refusal, reduction in nutrient absorption and metabolism, digestive disorders including haemorrhage and necrosis. Toxicity occurs at the cellular level. AFB₁ causes DNA changes, cell deregulation, cellular changes and death [20]. When AFB₁, the major toxic member of the group, contaminates the feed of lactating animal, the milk from these animals will contain aflatoxin M₁ (AFM₁), the principal metabolite arising from biortransformation of AFB₁. The presence of AFM₁ in milk can be a potential threat to the health of consumers of dairy products [5].

To reduce the risk of exposure, many countries have regulated the levels of AFB₁ in feed (and have set or proposed maximum permissible levels of AFM₁ in milk). Currently, the legal limits of AFB₁ in feedstuffs are highly variable from the European Union (EU) Countries to other countries (the EU has a limit of 5 µg/kg for dairy feed) [15].

The purpose of this study is to give the information of the occurrence of AFB₁ in dairy feed over ten years of observation.

### Table 1. Concentration and frequency of occurrence of AFB₁ in dairy feedstuffs over 10 years in Portugal

<table>
<thead>
<tr>
<th>Years</th>
<th>N/N (%)</th>
<th>1-5.0</th>
<th>5.1-10</th>
<th>10.1-20</th>
<th>20.1-80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N'</td>
<td>%</td>
<td>Mean</td>
<td>N'</td>
<td>%</td>
</tr>
<tr>
<td>1995</td>
<td>7/33 (21.2)</td>
<td>3</td>
<td>9.1</td>
<td>1.6</td>
<td>4</td>
</tr>
<tr>
<td>1996</td>
<td>11/29 (38)</td>
<td>10</td>
<td>34.5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1997</td>
<td>8/25 (32)</td>
<td>4</td>
<td>16</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td>1998</td>
<td>8/15 (36.6)</td>
<td>4</td>
<td>26.7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1999</td>
<td>9/149 (23.1)</td>
<td>61</td>
<td>42</td>
<td>3.5</td>
<td>23</td>
</tr>
<tr>
<td>2000</td>
<td>76/151 (50.3)</td>
<td>68</td>
<td>45</td>
<td>2.6</td>
<td>5</td>
</tr>
<tr>
<td>2001</td>
<td>50/139 (36)</td>
<td>48</td>
<td>34.6</td>
<td>2.2</td>
<td>1</td>
</tr>
<tr>
<td>2002</td>
<td>13/109 (12)</td>
<td>10</td>
<td>9.2</td>
<td>2.1</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td>78/180 (43.3)</td>
<td>78</td>
<td>43.3</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>26/175 (14.9)</td>
<td>26</td>
<td>14.9</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>374/1001 (37.4)</td>
<td>312</td>
<td>31.2</td>
<td>2.2</td>
<td>37</td>
</tr>
</tbody>
</table>

N: number of samples; N+: positive sample; samples < 1 µg/kg were considered negatives.
The recoveries were done in duplicate, in blank dairy cattle feed samples of (1 kg), spiked with level of 4.0 mg/kg of AFB1. The average recoveries was 98.0%. Confirmation of AFB1 was performed by thin layer chromatography (TLC), according to the method prescribed by Przybylski [18] and by Gimeno [3]. The identification of AFB1 is based on derivation of AFB1s, by reaction with trifluoracetic acid (TFA) (Sigma T-6508), directly on the TLC plate SIL G-25 HR (Macherey-Nagel, ref. 809033, Germany). Samples with AFB1 levels below 1 mg/kg were considered as negative (less than the detection limit).

The results concerning to the 2 determinations of each sample are summarised in Table 1. Three hundred and seventy four samples out of 1001 (37.4%) analysed samples for the presence of AFB1, were positive.

The level of contamination for AFB1 was generally low and ranged from 1µg/kg to 74 µg/kg. Levels of aflatoxin B1 above the maximum limit established in Portugal (5 µg/kg) for dairy cattle feed samples were observed in 62 samples (6.2%) with levels ranging from 5.1 to 74 µg/kg. Out of those 62 samples, 3.7% had levels between 5.1 to 10 µg/kg (mean 7.8); 1.8% had a contamination level of 10.1 to 20 µg/kg (mean 12.0), and 0.7% exceeded 20.1 µg/kg (mean 50.4). On the last two years (2003-04) none of the samples exceeded the maximum permitted level of the toxin.

Similarly to the present study, in 1980, Patterson and Roberts [17] reported a survey over a period of 13 years on the presence of AFB1 in 740 samples of animal feedstuffs, including dairy feed, where the toxin was detected in 27% of the dairy feed samples, at levels of more than 30 µg/kg. Osiwiler and Trampel [16] found AFB1 in the same type of samples in concentrations from 0.096 to 1.700 mg/kg. Skjinjar et al. [19] detected this toxin in dairy feed samples at levels ranging from 0.005 to 0.016 mg/kg.

According to Huni et al. [7] in Swiss commercial dairy feeds the AFB1 content decreased sharply from 47 µg/kg in the winter of 1976-77 to 24 µg/Kg in the winter of 1977-78 and to 3 µg/Kg in the winter of 1978-79 and this tendency was associated to the restrictive Federal measures adopted in this country. Similarly, the present study shows that from the year 2000, there is a decrease on the concentration of the toxin found in the dairy feed samples and since 2003 none exceeded the maximum permitted level of the toxin. This tendency is probably related to increasing surveillance measures on the sanitary quality of raw materials and the subsequent feedstuffs observed in Portugal in the last years [11].

Although there are some reports on the presence of moulds, and particularly A. flavus in feedstuffs in Portugal [12,13] the occurrence of the toxin produced by this fungus is scarcely referred. In 2001 Martins and Martins [13], observed the contamination of moulds (A. flavus) in 27% of 155 bovine feed samples during the years 1996 to 1999. Albeit these data might indicate problems in the quality of the feed, correlation with the presence of AFB1 is not possible to establish as not all the A. flavus strains are toxigenic [10].

Based on the results obtained in this study, a permanent monitoring for AFB1 by the feed industry, as well as awareness on the part of breeders is highly recommended and beneficial, since this mycotoxin affects animal’s health and the contaminated milk can be a serious public health hazard.