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Surveillance of aflatoxin content in dairy cow feedstuff from Navarra (Spain)

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ABSTRACT

Aflatoxins (B_1, B_2, G_1 and G_2) are produced by the fungi *Aspergillus* (*A. flavus* and *A. parasiticus*) in substrates used in cattle feed manufacturing. Aflatoxin M_1 (AFM_1) is a major metabolite of Aflatoxin B_1 (AFB_1) which may be present in milk from animals that consume contaminated feed. Levels of aflatoxins in 78 dairy cow feedstuff samples from 40 farms located in Navarra were determined by HPLC-FLD (High Performance Liquid Chromatography with fluorescence detection) and post-column derivatization. The influence of geographical location, season and type of feeding system on aflatoxin content was studied. The climatic profile of AFB_1 pointed to spring as the season with the highest aflatoxin level (0.086 μg/kg), followed by winter and summer (0.075 and 0.030 μg/kg, respectively), and to a lesser degree, autumn (0.017 μg/kg). Moreover, wet and dry TMR (Total Mixed Ration) feeding systems (*i.e.* AFB_1: 0.076 and 0.068 μg/kg; Aflatoxin G_1 (AFG_1): 0.050 and 0.011 μg/kg, respectively) showed a greater content of the analyzed aflatoxins in comparison with compound feed (*i.e.* AFB_1: 0.039 μg/kg; AFG_1: 0.007 μg/kg). The fact that the majority of the samples collected were based on compound feed shows that this type was preferred by most dairy farmers. The undetectable levels of aflatoxins in the organic homemade compound feedstuff are also worth mentioning. While none of the feedstuff samples contained amounts over those permitted under European legislation (5 μg/kg), the theoretical extrapolation of the carryover rate suggested in previously published experiments of AFB_1 to AFM_1 in secreted cow's milk predicts that only one of the feed samples studied had a positive aflatoxin level (53.4 ng/kg) higher than the legal limit for raw cow's milk.

1. Introduction

Mycotoxins constitute a potential threat to international public health (Méndez-Albores et al., 2007) because of their frequent occurrence in foodstuffs for humans and animals. These compounds are a heterogeneous group with very diverse origins. Aflatoxins (AFs) are produced by mainly *Aspergillus flavus* and *Aspergillus parasiticus*. These mycotoxins may occur

Abbreviations: AFs, Aflatoxins; AFB_1, Aflatoxin B_1; AFB_2, Aflatoxin B_2; AFG_1, Aflatoxin G_1; AFG_2, Aflatoxin G_2; AFM_1, Aflatoxin M_1; IARC, International Agency for Research on Cancer; CONTAM, Scientific Panel on Contaminants in the Food Chain; ME, metabolizable energy; GE, gross energy; *q*, ration diet quality; TMR, total mixed ration; FAPAS, Food Analysis Performance Assessment Scheme; n.d., non-detected; PBS, phosphate buffered saline; PVDF, polyvinylidene fluoride; HPLC, high performance liquid chromatography; Q_1, first quartile; Q_3, third quartile; HACCP, hazard analysis critical control points; GMP, good manufacturing practices.

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during harvesting, storage (and transport), production technology, processing and preparation of food. Moreover, the occurrence of AFs is enhanced by several factors such as stress due to drought before harvesting, insect activity, soil type and inadequate storage conditions.

There are more than 20 distinct, but structurally related, aflatoxin compounds. Aflatoxin B_1 (AFB_1), Aflatoxin B_2 (AFB_2), Aflatoxin G_1 (AFG_1) and Aflatoxin G_2 (AFG_2) appear in many food products, but especially in those with a high carbohydrate and lipid content such as nuts (peanuts, pistachios, walnuts), dried fruits (figs), cereals (maize), spices (pepper), seeds, cocoa and beer, as a result of fungal contamination before or after harvest (Garrido et al., 2012; Oruc et al., 2006). Most of the other AFs described in the reference literature come from hydroxylation at different points in the molecular structure of these AFs. In this respect, aflatoxins M_1 (AFM_1) and M_2 (AFM_2), 4-hydroxy derivative of AFB_1 and AFB_2 respectively, are found in mammals secretions (urine and milk). AFM_1 mammary excretion begins approximately 12–24 h after animals have ingested AFB_1 contaminated food and disappears about 24–72 h after its absence in the diet (Zinedine et al., 2007a).

AFs are extremely toxic: these compounds are immunosuppressive, mutagenic, teratogenic and carcinogenic in most organisms. The International Agency for Research on Cancer (IARC) has classified AFB and AFG in group 1 as human carcinogens, the liver being the main target organ for toxicity (IARC, 2012; Zain, 2011; Giray et al., 2007).

The transformation of AFB_1 from feedstuffs to AFM_1, consumed by cows, and subsequently carried over into secreted milk, depends on several feed-related factors (quantity, characteristics of the food consumed and the dose level of AFB_1), metabolism (milk yield, lactation stage, species, breed, time of day) and other factors such as weather and/or geographical location of dairy farms (Masoero et al., 2007). Taking into account all these relevant considerations, the predicted rate of AFB_1/AFM_1 carry-over from feedstuff into milk is approximately 0.3–6.0% (Heshmati and Milani, 2010). Van Eijkeren et al. (2006) proposed a steady-state model for predicting the correlation between AFB_1-contaminated feedstuff consumed by a dairy cow and AFM_1 excreted into milk.

Due to the toxicity of AFB_1, Directive 2002/32/EC provided a limit for undesirable substances in animal feedstuffs with 12% moisture content, setting an upper limit of 5 μg/kg for AFB_1 in complete feedstuffs for dairy cattle (EC, 2002). In addition, the EFSA CONTAM Panel (Scientific Panel on Contaminants in the Food Chain) has recently concluded that the currently established maximum levels for AFB_1 in animal feed not only provide adequate protection from adverse health effects in target animal species, but more importantly, appear to successfully prevent undesirable concentrations of AFM_1 in milk. Therefore, there is no need to modify the existing maximum levels for AFB_1 (EFSA, 2004).

However, the occurrence of different AFs in animal diet during its production or storage is quite heterogeneous and depends on many factors: the environmental conditions during fungal growth, the different feeding patterns depending on the season, agricultural practices, *etc.* It therefore seems reasonable to ask for closer surveillance and monitoring of food products, cereals and fodder for animal consumption (Signorini et al., 2012).

At this respect, the AFs production is not particularly restricted to any ingredient of the animal feeding but the AFs levels vary, as mentioned above, with location and climatic profile which determine the risk of contamination in the dairy cow feeding (Bryden, 2012). As the aflatoxin-contaminated dairy cow feed is intrinsically related to a deficient dairy farming, any threat to feed security could involve a significant impact on the economic vitality of the dairy cow farm (Cheli et al., 2013). Cow milk farmers have often attempted different strategies to reduce feed costs. The evaluation of the cost-effectiveness of different types of dairy cow feeding systems is a common practice. In this regard, the total mixed rations (TMRs) are widespread based on economics and practicality. Nonetheless, an adequate choice of the dairy cow feeding system is crucial to avoid the potential risk of aflatoxin contamination of feedstuffs, contributing with a negligible aflatoxin exposure of the dairy cows fed on. Therefore, a complete description of different feeding systems based on AFs content will be useful to provide satisfactory data for dairy cow farmer to develop a traceability system with the purpose of minimizing a potential hazardous exposure. Taking into account these points, the rationale for the current work is the assessment of the AFs concentration levels supplied by different dairy cow feeding systems: (i) based on compound feed (conventionally and organically produced) supplied together with alfalfa, hay and straw to complete the TMR; (ii) wet- and (iii) dry-TMR feeding systems combining all forages, grains, protein feeds, minerals, vitamins and feed additives, manufactured with different moisture. As it is evidenced by their qualitative composition, all studied feeding systems might supply a similar source of aflatoxin contamination. Hence the similarity in these compounds allows a helpful statistical comparison of different groups of cow feedstuff in relation to the well-known factors of mycotoxin contamination (Driehuis et al., 2008; Cheli et al., 2013). Specifically, the aims of the present study are to evaluate: (a) the occurrence of aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) in different dairy cow feedstuff samples; (b) the potential relationship between the degree of contamination with these mycotoxins and the influence of seasonal factors, geographical location and animal feeding systems; (c) to assess the exposure of dairy cattle to AFB_1; and (d) to estimate, based on the theoretical intake, its biotransformation into AFM_1 and the subsequent carryover into raw cow's milk.

2. Materials and methods

2.1. Dairy cow feeding sampling

The animal feed study was carried out in 2008 in collaboration with the Danone cow milk collection center (Ultzama, Navarra, Spain); and included several dairy farms from five different sampling areas (Baztán: 43.15°N, 1.50°W;