Aflatoxin B1 risk management in *Parmigiano Reggiano* dairy cow feed

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Abstract

This study investigated aflatoxin B1 (AFB1) contamination in dairy cow feed and the risk management of AFB1 content in concentrates undertaken by feed industries in the *Parmigiano Reggiano* area. Data on aflatoxin contamination risk management applied in 29 feed industries were collected and the AFB1 content of 70 feed samples was analysed. Data were collected within the framework of a quality control programme promoted by the *Parmigiano Reggiano* Consortium in 2013 and 2014. Audit results showed that the control procedures to prevent AFB1 contamination mainly focused on maize and its by-products. AFB1 concentration resulted lower than 5 ppb [legal European Union (EU) limit] in all samples; in one out of 70 samples, AFB1 content was 3.8 ppb and in all the other samples it was lower than 3 ppb. Results showed that AFB1 risk management applied by Italian feed industries effectively monitors AFB1 levels in feed below the EU legal limit.

Introduction

Aflatoxins are secondary metabolites produced by different fungi of the species *Aspergillus*, mainly *A. flavus* and *A. parasiticus*, but also *A. nominus* and *A. tamarii* (Kurtzman *et al*., 1987; Goto *et al*., 1997), the strain *A. pseudotamarii* isolated by Ito *et al.* (2001), and also *A. niger, A. wentii, Penicillum citrinum* and *P. frequentans* (Caloni and Nebbia, 2009). These organisms may contaminate agriculture products, feedstuff and food. The aflatoxin group includes aflatoxin B1 (AFB1), B2, G1 and G2, and their hydroxylated metabolites can be found in milk. Aflatoxins M1 (AFM1) and M2 are the metabolites of AFB1 and B2 respectively. AFM1 is the most commonly occurring aflatoxin detected in milk of mammals exposed to AFB1 ingestion. AFB1 is classified as Group 1 (carcinogenic to humans) while AFM1 is classified as Group 2B (possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC) (Caloni and Nebbia, 2009).

Aflatoxin contamination risk is generally higher in geographical regions with a tropical or subtropical climate, but the abnormal hot and drought-growing seasons of maize have raised problems in crop management especially in Southeast Europe (Serraino *et al*., 2003; Trevisani *et al*., 2014). Maize grain, which is normally used to feed dairy cattle, is produced with the use of irrigation systems in this region. Maize can be infected by moulds when hot and dry environmental conditions occur during the pre-harvest phase, and weeds and crop disease contribute to grain contamination by aflatoxin (Giorni *et al*., 2007; Prandini *et al*., 2009; Kebede *et al*., 2012). In 2003 and 2012, unfavourable weather conditions, such as temperatures above 30°C and the lack of precipitation over an extended period, caused a massive contamination of maize in Northern Italy resulting in an increase in AFM1 in cow's milk (Canever *et al*., 2004; Marchetti *et al*., 2013).

The carryover of aflatoxin from cattle feed to milk depends on the animal breed, the lactation period and udder infections (Masoero *et al*., 2007). The average value is approximately 2.5%, but a direct relation between carryover rate and milk yield was found with carryover percentages as high as 6.2% in high-yielding dairy cows with a production of up to 40 L of milk per day (Veldman *et al*., 1992). Despite the high individual variability, the equation proposed by Veldman *et al*. (1992) is used to estimate the carryover of AFM1 in a whole dairy herd:

$$\text{AFM1 (ng/kg milk)} = 1.19 \times \text{AFB1 (µg/cow/day)} + 1.19$$

Using this equation is it possible to assume that, in order to obtain a concentration of AFM1 below the maximum limit allowed by EU Regulation 1881/2006 (European Commission, 2006), the average intake of AFB1 must be less than 40 µg/day. Considering that a complementary feed can be given to a dairy cow at levels of >10 kg/day and that its aflatoxin contamination limit is 5 ppb (EC Regulation 574/2011; European Commission, 2011), it is easy to deduce that it is possible produce non compliant milk even feeding cows with compliant (5 ppb) complementary feed. For this reason several integrated dairy chains require to the feed industry a lower AFB1 content in their products and they provide a better information for dairy farmers. In 1993 the *Parmigiano Reggiano* (PR) Consortium signed a protocol with feed industries to reduce the AFB1 content in concentrates for dairy cow feed to a level below 3 ppb instead of the 5 ppb EU limit.

This work reports the results of an investigation, performed in the *Parmigiano Reggiano* area, on AFB1 contamination in dairy cows complementary feed and on the related risk management implemented by feed industries.

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Key words: Aflatoxins; Feed; Dairy cows; *Parmigiano Reggiano.*

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 26 May 2015.
Revision received: 24 June 2015.
Accepted for publication: 24 June 2015.

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Licensee PAGEPress, Italy
Italian Journal of Food Safety 2016; 5:5291
doi:10.4081/ijfs.2016.5291

Materials and Methods

During 2013 and 2014, in the framework of a quality control program performed by the PR Consortium (Ricci *et al*., 2015), data on AFB1 risk management were collected in 29 feed industries. The quality control program was based on a voluntary agreement between the feed industries and the PR Consortium. The agreement considered the application of requirements defined by the specification of *Parmigiano Reggiano* cheese (for example a ban on the use of cotton seed, canola and others feedstuffs) and additional requirements defining higher processing and composition standards compared to EU Regulation n° 574/2011 (European Commission, 2011). The additional requirements regard the aflatoxin content (fixed at 3 ppb instead of 5 ppb),

absence of gossypol, absence of volatile mustard oil, the implementation of procedures to avoid cross-contamination with prohibited raw materials like rice, rapeseed and cotton in dairy cow feed.

Audits were performed twice in each of the 29 feed industries, once in 2013, collecting one feed sample in each plant (totally 29 samples) and the second in 2014 collecting 41 feed samples, more than one in each plant.

Samples collected in 2013 were analysed for the quantitative detection of AFB1 by Ridascreeen® Aflatoxin B1 30/15 Biopharm kit (Darmstadt, Germany) whereas the samples collected in 2014 were first screened for total Aflatoxin content by I'Screen Afla (Tecna, Trieste, Italy) and AFB1 was quantified only in samples with an Aflatoxin B total content higher than 3 ppb by a LC-MS/MS method (Biancardi and Dall'Asta, 2014).

Results

Audit results showed that the AFB1 contamination risk management applied by feed industries mainly focused, as expected, on maize and its by-products. Procedures performed by feed industries to reduce AFB1 contamination of maize included: i) purchase of maize in geographic areas with a low AFB1 contamination risk due to favourable environmental conditions or to a particular organisation of the maize supply chain; ii) specific commercial agreements with suppliers that included a maximum limit of AFB1 concentration; iii) a high frequency (up to 100%) of delivered maize analysis; iv) the selection of maize on the basis of AFB1 content and differentiated use of AFB1 in feed for different species (for example low AFB1 contaminated maize for dairy cow feed and high AFB1 contaminated maize for swine feed); v) high frequency of maize purchasing reducing storage time in the feed mill. More details on AFB1 risk management are reported in Table 1. The AFB1 content was <2 ppb in 28 out of 29 samples (96.6%) collected during 2013 and 3.8 ppb in the last sample. In 41 samples collected during 2014, the AF total content was below the limit of detection (2 ppb), in the range 2-3 ppb, and >3 ppb (up to 4 ppb) in 14 (34.1%), 24 (58.5%) and 3 (7.3%) samples, respectively. The 3 samples showing aflatoxin contamination >3 ppb were subsequently analysed by LC-MS/MS and AFB1 concentration was <1 ppb in all these samples.

Discussion

Few data are available on feed materials originating from Europe (European Food Safety Authority, 2004) as aflatoxin formation was previously considered to occur mainly in geographic regions with a tropical or subtropical climate. On the other hand, AFB1 contamination of feed and feed ingredients, mainly maize, appears to be a problem in southern Europe, and the contamination level is strongly influenced by climate conditions. Out of 533 maize samples for animal feed collected in Northern Italy during the period 1995-1999, Pietri *et al*. (2004) reported high positive rates (42.9%), although levels were generally very low (mean concentration between 1.3 and 5.1 ppb during years) with the exception of 2 samples contaminated with AFB1>100 ppb and 5 samples >20 ppb. Minervini *et al*. (1998) analysed 197 dairy cow feed samples and found all of them <5 ppb and another 124 maize samples where only 9 showed values between 5 and 20 ppb, with no samples >20 ppb. In the Lombardy region (1999-2000) 830 dairy cows feed samples were analysed and all feedstuffs showed AFB1 levels <5 ppb (Amodeo, 2001).

On the contrary, during the 2003 crisis, several feed samples were analysed in dairy farms where AFM1 milk contamination was above the legal limit of 50 ppt. The results showed that 58.9% of complementary feeds and from 14.48 to 66.66% of maize and maize products exceeded the EU legal limit (Canever *et al*., 2004). A study performed from the beginning of 2004 to the end of 2005 reported that AFB1 in cattle feed was higher than the maximum limit in 8.1% of feed samples, whereas during 2005 found aflatoxin levels constantly below the limits of EU regulations (Decastelli *et al*., 2007). More recently, a large 2012 survey conducted on corn produced in Northern Italy analysed 31,326 samples collected at storage plants. The results showed that AFB1 contamination above the EU limit of 20 ppb was detected in samples representative of about 784,000 tons of corn, corresponding to 45.2% of the total production (Causin, 2013).

The results of the present study showed that no sample had AFB1 levels above the EU legal limit of 5 ppb in the investigated feed mills. Moreover, auditing showed that a good level of AFB1 risk management has been achieved by Italian feed industries to counteract the unfavorable climatic conditions arising in some years. The special attention paid by the feed industry to maize AF contamination is probably the result of the two seasonal crises in 2003 and 2012 when unfavorable climatic conditions led to a massive AFB1 contamination of maize with up to 50% of national maize production non-compliant with EU Regulations and unsuitable for feeding milking cows (Marchetti *et al*., 2013). As a consequence, monitoring was stepped up by all the dairy production chains, including feed manufacturers and health authorities, also thanks to the availability of rapid and less expensive detection kits to test aflatoxin contamination in raw materials. No limit is reported for AFM1 in cheeses in the European Community legislation, but the provisional limit of 450 ppt was fixed for hard and long maturing cheeses by the Italian Ministry (Italian Ministry of Health, 2004). An investigation performed on *Grana Padano* cheese produced with naturally contaminated milk showed that AFM1 concentration levels increase 4.5-fold in cheeses (Manetta *et al*., 2009). This guarantees that the level of AFM1 contamination of cheese will be lower than the 450 ppt limit even when milk with an AFM1 contamination above the legal limit (50 ppt) is used.

Table 1. Actions taken by twenty-nine feed industries to reduce maize aflatoxin B1 contamination in 2013 and 2014.

Type of action	Feed industries implementing the action (n) Year 2013	Year 2014
Testing 100% of maize consignments	22	21
High frequency (below 100%) of delivered maize analysis	2	4
Definition of specific commercial agreements with suppliers	7	6
Maize purchase in geographic areas with low risk of AFB1 contamination	1	0
Selection of maize on the basis of AFB1 content and its differentiated use in feed for different species	1	3
High frequency of maize purchasing and reduction of storage time in the feed mill	7	12

AFB1, aflatoxin B1.

Conclusions

The application of a voluntary agreement to reduce the AFB1 contamination of feedstuffs further reduces the health risk for consumers.

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