



## An Update on the Safety of Foods of Animal Origin and Feeds

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## PAPER

## An update on the safety of foods of animal origin and feeds

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### Abstract

Chemical hazards may occur in any phases of the different livestock production systems. Aim of this review is to address an update about the key issues related to the risk of contamination in foods of animal origin by environmental contaminants linked to industrialisation or urbanisation (*e.g.*, heavy metals and persistent organic pollutants), and natural contaminants (*e.g.*, mycotoxins). This review deals with current issues and future perspectives on the complex issue of the safety of feeds and foods of animal origin, by taking into account the estimation of the occurrence of chemical residues in food, the hazard identification and characterisation of mycotoxins in animal feeds, and the analysis of feedstuffs as a tool to control and evaluate food safety.

### Introduction

Production of safe and healthy foods is a major challenge for both developed and developing countries. Since the beginning of this century, the European Union (EU) has launched an extensive food safety programme,

based on the report *White Paper on Food Safety* presented by the Commission of the European Communities (European Commission, 2000), with the following starting point: Assuring that the EU has the highest standards of food safety is a key policy priority for the Commission. This White Paper reflects this priority. A radical new approach is proposed. This process is driven by the need to guarantee a high level of food safety. More than ten years after, the application of the White Paper principles seems to have reached many of the EU goals, as evidenced by the institution of an independent European Food Safety Authority, the identification of a wide range of measures that are necessary to improve food safety standards, the improvement of food safety control, the increase in consumers information, and the accounting of the international dimension of the problem. Nowadays, European citizens are more informed about and better protected by the authorities against the risks of natural and artificial food, thanks to the great work carried out by the European Food Safety Authority (EFSA) (Byrne, 2013). On the other hand, the issue of food safety has become increasingly more important because of the greater complexity of the food system and its openness to international markets, the improvement of monitoring technologies and the emerging risks related to climate change. This is especially true for animal products, which represent about 43% of EU outputs from agriculture (Eurostat, 2014).

This review deals with current issues and future perspectives on the complex issue of the safety of feeds and of foods of animal origin, by taking into account i) the estimation of the occurrence of chemical residues in food, ii) the hazard identification and characterisation of mycotoxins in animal feeds and iii) the analysis of feedstuffs as a tool to control and evaluate food safety.

### Estimation of occurrence of persistent, bioaccumulative and toxic chemicals residues in food

Assuring a safe environment and high-quality and safe food is essential to promote long-term development and human health and welfare. Good environmental quality is a focal point of the Lisbon Agenda, the European Council of Goteborg and the Sixth Environment Action Programme (6<sup>th</sup> EAP), which is the EU's ten-year policy programme

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for the environment. In Goteborg, the European Council (2001) agreed on the priority of the EU policies and highlighted the strategies to face the public health threats. The Goteborg Council also underlined how the EU should give adequate answers to the citizens' concerns about the safety of food resources and the use of chemical substances. Overall, there is a growing concern about the potential impact of chemicals on human health and environment. In particular, the exposure to persistent and bio-accumulative organic compounds, endocrine-disrupting chemicals (EDCs) and heavy metals are thought to be linked with declining sperm counts, genital malformations, impaired neural development and sexual behaviour, obesity and cancer (European Environment Agency, 2012).

### Persistent organic pollutants and livestock production systems

Nowadays, the main concern regarding the so-called persistent organic pollutants (POPs) and undesired or toxic trace elements (heavy metals) is the indirect environmental contamination following the accumulation of wastes in industrial areas. In Europe, a large number of contaminated sites exist and, recently, the European Environment Agency (EEA) estimat-

ed that at least 250,000 sites are at risk, with approximately 2.4% of them representing a potential hazard for the ecosystem (European Environment Agency, 2007). Agro-ecosystems have been threatened by the presence of uncontrolled contaminated sites, with several cases of contamination being recorded, especially in Eastern European countries. In Italy, recent estimates made by non-governmental organisations showed that there are more than 12,500 potentially hazardous sites, mainly distributed in the northern part of the country (6729 sites) (Federambiente, 2010), where a large part of the meat, milk and eggs are produced. In the same area, a number of urban-waste thermal-treatment plants (Federambiente-ENEA, 2009) and carbon-burning power plants (Assocarboni, 2013) may seriously damage the environmental quality of the neighbouring agro-zootechnical systems.

Some chemicals can be transported and accumulated in the soil resulting in contamination of water, foodstuffs of plant origin, fodder and feeds. The migration of contaminants through the food chain may have a negative impact on the health of the indigenous wildlife, farm animals and, finally, humans (Olivero-Verbel *et al.*, 2011; Mrema *et al.*, 2013).

The so-called EDCs, which represent a serious risk for the safety of food products, are substances of natural or man-made origin that are able to alter the proper functioning of the endocrine system in animals and humans (Toppari *et al.*, 1996; Safe, 2005; Mrema *et al.*, 2013). Among the EDCs, the organochlorinated pesticides (OCPs) are a group of man-made chemicals characterised by the presence of organic rings substituted with chlorine atoms. These pesticides are very stable in the environment, because they are not easily metabolised, and are lipophilic, thus tending to accumulate at high amounts in fat tissues, human and animal milk, meat and eggs (Olivero-Verbel *et al.*, 2011). The OCPs, such as the hexachlorocyclohexane isomers (HCHs; mainly the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -HCH), have been widely sold and used in developing countries for crop protection (Iwata *et al.*, 1993), being released in the environment at rates often incompatible with the natural degradation processes. Many European countries severely restricted or banned the use of HCHs between 1977 and 1992 (Breivik *et al.*, 1999). The United States banned the use of technical HCH for agricultural purposes in 1976 (Agency for Toxic Substances and Disease Registry, 2005), and have revoked all authorisations for plant protection products containing Lindane (pure  $\gamma$ -HCH) since 2001.

In 2005, there was a case of agro-environmental contamination by HCHs in the Sacco River Valley (Province of Rome, Lazio Region) in Italy, where HCH isomers were found in the soil, plants and bovine milk in a large rural area with dairy cow farming (Ronchi and Danieli, 2008). The occurrence of these contaminants was associated with the presence of a chemical industry born in the early 900s that produces chemicals such as OCPs, phosphate esters and ketones, the intense industrial production of explosives, railway carriages and wagons, and the uncontrolled disposal of processing residues and unsold fine chemical stocks in some unauthorised landfills. In a case study conducted in the Sacco River Valley, all the components of the dairy cow production system subjected to the agro-environmental pollution crisis were investigated in five dairy farms in 2006, to evaluate its main critical points. Data regarding the contamination of soils, forages, bovine milk and bovine blood serum by HCHs were reported. Soil and forage samples (mainly corn, alfalfa and ryegrass) were collected in different places around the Sacco River, varying for irrigation practices and flooding conditions, and were analysed by Gas Chromatography using an Electron Capture Detector. Soil contamination by HCHs was higher near than away from the river ( $P < 0.01$ ), with a great incidence of outflow risk ( $P < 0.01$ ), whereas it did not differ between irrigation practices. The alfalfa samples had a higher concentration of HCHs than the ryegrass samples, with a greater plant/soil apparent partition factor. Differences in milk contamination by beta-HCH among dairy farms ( $P < 0.01$ ) and sampling times ( $P < 0.05$ ) were found. In many cases, the beta-HCH content in milk was above the EU limit of 0.003 mg  $\beta$ -HCH/kg set by the Reg. 149/2008 (European Commission, 2008), posing a serious risk for human consumption due to the chronic toxicity of that isomer. Differences in milk beta-HCH concentration were related to lactation phase and parity, with the highest levels of beta-HCH being found in milk produced by pluriparous cows in the first lactating phase (before 100 days in milk). A linear regression between blood serum and milk beta-HCH concentration was observed ( $r^2 = 0.92$ ,  $P < 0.05$ ). Furthermore, beta-HCH was detected at trace levels in blood sera when its levels in milk fell below the analytical limits. As pointed out by other authors (Gupta *et al.*, 1978, To-Figueras *et al.*, 1997, Otero *et al.*, 1997, Waliszewski *et al.*, 2004), the results of the study reported by Ronchi and Danieli (2008) suggest a high sensibility of blood as an early indicator of OCP exposure, thus making cattle blood sampling an useful

technique in biomonitoring plans to prevent animal exposure to agro-environmental pollution. The pollution by polychlorodibenzodioxins and -furans (PCDDs and PCDFs) and dioxin-like polychlorobiphenyls (DL-PCBs) of food of animal origin can give an useful indication in determining the link between livestock and the territory contamination, especially for extensive and outdoor farming systems. Environmental quality standards able to support food safety/food security have been explored in extensive farmed sheep and pigs within area of Goceano in Sardinia (Brambilla *et al.*, 2011a) and the Regional Parks of Nebrodi in Sicily (Brambilla *et al.*, 2011b), respectively. Such locations have been selected because far from the pressure of known anthropogenic and industrial sources of environmental emission of selected lipophilic POPs, such as PCDDs/Fs, DL-PCBs, and Polibromodiphenyl ethers (PBDEs). Outdoor reared pigs and sheep were considered as sentinel of the overall environmental quality of the Mediterranean landscape: their grazing behaviour determines a relevant top soil intake, thus influencing the levels of the aforesaid contaminants in edible tissues. Liver, as most bioaccumulating organ, fat associated to muscle, and milk were considered for the analysis via high resolution gas chromatography coupled with high resolution mass spectrometry (HGC-HRMS). Results indicated that pastures impacted by natural fires during the summer season represent the most relevant source of POPs intake in animals, possible leading to milk contamination ( $> 2$   $\mu\text{g WHO-TE/g fat}$ ), above the regulatory action level for PCDDs/PCDFs and DL-PCBs in sheep milk. On the contrary, where the natural landscape was preserved, the observed contaminations ( $< 0.7$   $\mu\text{g WHO-TE/g fat}$ ) felt below the average values inventoried in the same item from intensive farmed animals (Brambilla *et al.*, 2011a). Similarly, outdoor reared pigs in preserved environmental area showed background contaminations (0.05 and 0.78  $\mu\text{g WHO-TE/g fat}$  in muscle and liver, respectively) against the 0.45 and 12.7  $\mu\text{g WHO-TE/g fat}$  in the same matrices from wild animals grazing in areas affected by bushfires. Results from PBDE analysis were in the same direction, thus suggesting the preservation of the environmental quality in natural landscape represents a critical factor to ensure both food safety and food security in small scale, extensive and organic farming systems (Brambilla *et al.*, 2011b). Such an environmental support may improve the impact of the rural and organic farming on socio-economics, because the added economic value of the organic food retains in the local communi-

ty, but also for the beneficial effects on other activities, such as tourism, to create greater economic opportunities in a wider community.

### Heavy metals and safety of animal food products

Metals make up three-fourths of the elements in the periodic table, but only few of them are essential for life. Most of the known metals are quite toxic to living organisms (Ballatori, 2002), being often named heavy metals (*e.g.*, lead, cadmium, chromium and mercury). These elements are ubiquitous in the environment, and their entrance into the food chain through soil, water and air circulation is an important environmental issue that entails risks to humans. Based on estimated loading rates of heavy metals into environmental compartments, human activity has a major impact on the global and regional cycles of heavy metals, accelerating their accumulation in the human food chain (Nriagu and Pacyna, 1988). The World Health Organization highlighted that from 60 to 80% of the body burden of toxic metals in people living in industrialised or urbanised areas is mainly caused by the intake of metals via food consumption rather than by inhalation of polluted air (Bellows, 1999). Widespread soil pollution is generally associated with atmospheric deposition processes, which are the result of industrial or agricultural practices, such as industrial emissions and spreading of sewage sludge, fertilisers and pesticides (European Commission, 2001; Nicholson *et al.*, 2003). Even though biologically relevant elements exhibit different properties in the soil, leaching losses and plant uptake are normally small if compared with total inputs. As a consequence, these potentially toxic elements may slowly accumulate in the soil profile over time (Nicholson *et al.*, 2003), with possible long-term implications for the quality of agricultural soils and the transfer of toxic elements to the human food chain from an increased crop uptake or soil ingestion by grazing livestock. Even with improvements in the treatment of the wastewater and sewage in agriculture (European Commission, 2001), these sources may contribute significantly to the total input of heavy metals in agricultural soils. According to recent estimates (European Commission, 2010), approximately 40% of the about 10 million tonnes of dry solids of sewage sludge produced in the EU-27 is recycled in agriculture. In the future there will probably be a general phase out of dumping sludge in landfills and an increase in sludge treatment (*e.g.*, anaerobic digestion and other biological treatments) before recycling it in agriculture. For this rea-

son, an increase in restrictions on the types of crops that can be cultivated using treated sludge is foreseeable to avoid excessive plant uptake and transfer of metals and other chemicals into the human food chain (European Commission, 2010). Beyond the concern about the increasing accumulation of heavy metals throughout the EU food chains, attention should be given to food imports from developing countries where the environmental protection rules are not well established. As a consequence of urbanisation, humans have created a distinct division between agricultural and city life (Flora, 2001), with most people today living far away from food production areas. However, this boundary is more conceptual than physical, because there are many areas of transition between farming and urbanised areas, such as rural acreages. In many European cities the boundary between cities and farms is clearly identified, but this is not true for other contexts, such as China, where a rural industrial development has recently occurred on a large scale (Xu, 1999). It is also true that the food and drink imports in the EU from China from 2000 to 2009 reached 3,1 billion € (+130%) (Confederation of the Food and Drink Industries in Europe, 2011). Similarly, in the same trading period, food imports into USA from China increased by about 9.5% per year, mainly because of the strong pressure to lower costs, leading to greater purchases of a wide range of products (*e.g.*, food, chemicals, drugs, machinery) especially from developing Countries (Food and Drug Administration, 2011). Overall, the human exposure to toxic elements depends on specific factors, such as age (young, adult or elderly), lifestyle, and food habits. For example, specific population groups may be exposed to environmentally relevant elements due to the consumption of meat of wild species (Taggart *et al.*, 2011; Danieli *et al.*, 2012). As far as the accumulation of heavy metals throughout the human food chain is concerned, lactating ruminants at pasture may be very useful to monitor the environmental occurrence of toxic elements such as Pb, Cd and Cr. The acute and chronic exposure to heavy metals may induce toxic effects in animals and humans, affecting particularly the neuronal, cardio-circulatory, urinary, immune, and reproductive systems. However, the Reg. (CE) No 1881/2006 in force set the contamination limit only for the presence of lead in milk (0.020 mg Pb/kg) (European Commission, 2006a). In a recent survey, Ronchi and Danieli (unpublished data) studied the occurrence of these metals in sheep milk collected in some areas of the Viterbo Province (Latium Region,

Italy), whose territory is mostly of volcanic origin (Vulsino and Cimino volcanic complexes). In the study area, there are some widespread emission sources (intensive agriculture) and several localised emission sources (ceramic manufacturers in C. Castellana, motorways, highways, the power plant of Montalto di Castro, urban waste landfills). In order to describe the occurrence of heavy metals (Pb, Cd, Cr, Zn and Cu) in sheep milk samples, to identify one or more environmental or managing factors influencing their occurrence and to assess the use of the lactating ewe as an *agro-environmental sentinel*, a sampling plan was designed to collect ewe milk from 9 sheep farms located within the North-West (N-W, 5 farms) and the South-East (S-E, 4 farms) areas of the Viterbo Province in 2002. A total of 25 samples of ewe milk were analysed by Atomic Absorption Spectrophotometry after oxidizing-acidic mineralisation. For Pb, 14 out of 25 samples were above the EU limit set for cow milk. Different factors were considered to explain the Zn, Cu, and Cr contamination of milk but a clear spatial dependence was found only for the Pb occurrence. In particular, the milk samples coming from farms located in the N-W area tended ( $P=0.052$ ) to be more contaminated by Pb than those from sheep farms located in the S-E area. Even though it was not possible to find an experimental relationship, due to the lack of heavy metal fall-out data, the localisation of the Power Plant of Montalto di Castro and the main wind direction existing in that area suggest a possible effect of the Power Plant activity on the level of Pb contamination found in sheep milk. In addition a quite good correlation was found between these data on sheep milk contamination and the data from a previous research (Danieli *et al.*, 2004) on heavy metal accumulation in cattle, goat and sheep offal (liver), gathered during official inspections carried out in the same area on dead animals following suspected intoxication ( $r=0.74$ ,  $P<0.01$ ; unpublished). Furthermore, estimates of Cd intake by lactating ewes based on literature data (Mehennaoui *et al.*, 1999) suggested that the safety threshold set for Cd in sheep feeding (National Research Council, 1980) might be exceeded. All these findings showed the presence of agro-environmental pollution by some heavy metals and that the ovine species could be a useful *agro-environmental sentinel* to investigate the time-spatial environmental burden due to heavy metal releases.

Agro-environmental pollution is one of the main challenges for the safety and quality of the Italian agro-food production, especially of animal food products, such as meat and

cheese. Considering the existing pollution sources (Federambiente-ENEA, 2009; Federambiente, 2010; Assocarboni, 2013; and the importance of the animal products for the national economic balance and consumers' health, further efforts should be made to solve this problem. In particular, a novel approach combining epidemiological data and new technologies for spatial analysis (*e.g.*, GIS, remote sensing, and satellite photo-interpretation) could be developed as an informative-operative tool for the assessment and management of environmental risks. In this respect, a GIS-based geo-statistical approach has recently given promising results for mapping risk of milk contamination by pesticides (Battisti *et al.*, 2013).

### Hazard identification and characterisation of mycotoxins in animal feeds

Mycotoxins are secondary metabolites produced by various genera of fungi growing on agricultural products. Some moulds can colonise grains before or after harvest or in both phases. However, the presence of fungi on a crop does not lead necessarily to the production of mycotoxins, and the occurrence of mycotoxins on feedstuffs does not imply necessarily their simultaneous presence with viable moulds.

Among feed ingredients, cereals (corn, sorghum, barley and wheat), cottonseed meal, groundnuts and other legumes are the main crops affected by mycotoxin contamination. These molecules are relatively stable, are not destroyed during feed processing and may even be concentrated in screenings.

Mycotoxins are very diverse in terms of chemical structure and toxic effects on animals. When the intake of mycotoxins from contaminated feed exceeds certain levels, adverse effects on livestock health might occur, such as reduction in growth performance or fertility, alteration of the immunity system, and, in cases of severe intoxication, death.

Among the 300-400 compounds now recognised as mycotoxins, only few of them are economically relevant, namely aflatoxins (AFs), trichothecenes, zearalenone (ZEN), ochratoxins and fumonisins (FBs). Recently, Streit *et al.* (2013) reported the results of an 8-year worldwide survey (2004-2011) on mycotoxin contamination of more than 17.000 samples of feed and feed raw materials. Seven out of 10 samples (72%) contained at least one mycotoxin, and about 4 out of 10 were contaminated with more than one toxin. More than half of the samples were contaminated with deoxynivalenol (DON) or FBs. Mycotoxin contamina-

tion was strongly affected by climate conditions. Although feeds were often highly contaminated with mycotoxins, the contamination levels were usually below the limits of the EU regulations or recommendations (European Commission, 2006b, 2011). In particular, 82% of the samples contaminated with AF did not exceed the 5 µg/kg limit for use in dairy animals, and 84, 85, 96 and 99% of samples did not exceed the lowest applicable guidance values for ZEN, DON, FBs and ochratoxin A (OTA), respectively.

### Mycotoxins in food of animal origin

The occurrence of mycotoxins in foods of animal origin may be the consequence of indirect contamination, because of the transfer from contaminated feeds, and direct contamination, as a consequence of mould growth on foods during processing, storage or aging. Therefore, a systematic approach to avoid that mycotoxins enter the food chain via animal products (*e.g.*, milk, meat and eggs) has to consider the risk of feeding livestock with contaminated feed. However, it is important to highlight that foods of animal origin contribute only marginally to the total human exposure to mycotoxins, because their carry-over from feeds into meat, milk or eggs is normally very low and was reported for only very few toxins.

So far, in the EU countries, the regulatory limits have been set up by the EC (European Commission, 2006a) only for aflatoxin M1 (AFM1) in milk and milk-derived products, whereas the risk management of other mycotoxins in the human food chain has been based on controlling the contamination of foods of plant origin and feedstuffs for livestock.

A maximum level of the undesirable aflatoxin B1 (AFB1) in animal feeds has been established by several countries, to prevent its adverse effects on animal health and to control the presence of its metabolite AFM1 in milk. In the EU, the maximum content of AFB1 in feeds set by the European Commission (European Commission, 2011) ranges between 0.02 and 0.005 mg/kg, with the lowest value being for feed for dairy and young animals (Table 1). Moreover, its member states have to ensure the application of the guidance values for the presence of DON, ZEN, OTA and fumonisin B1+B2 in feed materials and complementary and complete feedstuffs for animal feeding (European Commission, 2006b; Table 2). This regulatory action of EU is a consequence of previous reports of the EFSA showing that the carry over rate of DON, ZEN and FBs from feeds to products of animal origin is very low (European Food Safety Authority, 2004a,

2004b, 2005). Moreover, in animals fed OTA, this toxin occurs predominantly in kidney and liver, being much lower in milk, meat and eggs (European Food Safety Authority, 2004c). Actually, OTA can occur in meat and meat products as a result of indirect contamination from animals fed contaminated diets (Pietri *et al.*, 2006; Battacone *et al.*, 2010; Dall'Asta *et al.*, 2010; Duarte *et al.*, 2012). In an experiment carried out to assess the transfer of OTA in the pork product chain, Bertuzzi *et al.* (2013) reported that feeding pigs with diets containing slightly less than 50 µg/kg of OTA (that is the guidance value recommended by the EC) led to the consequent presence of the toxin in muscle at concentrations close to 1 µg/kg, which represents the guideline value for meat products recommended by the Italian Ministry of Health.

Because high OTA contamination in feeds is needed for its transfer into eggs, the consumption of this product of animal origin seems not to be a matter of concern regarding the intake of OTA, DON and ZEN by humans (Tangni *et al.*, 2009). When hens were fed diets containing 2 mg/kg of OTA, no OTA residues were detected in eggs (Denli *et al.*, 2008).

In ruminant species, the microbial activity of the microflora in the rumen is an efficient biological tool to dramatically reduce OTA bioavailability in the gastrointestinal tract through its hydrolysis to ochratoxin-alfa (Mobashar *et al.*, 2010). Although the presence of OTA in the milk of ruminants has been reported (Boudra *et al.*, 2007; Pattono *et al.*, 2011), this is not considered a relevant problem for the consumers because of the excretion of OTA in milk occurs only under certain conditions and the carry-over rate is very low.

In the early sixties of the last century, researchers reported that mammals (rat and cow) were able to convert AFB1 into a milk toxin, later named aflatoxin M1 (AFM1), and that the presence of this toxic metabolite in the milk was a consequence of AFB1 intake (Allcroft and Carnaghan, 1963; De Longh *et al.*, 1964). During the last five decades, numerous studies have evaluated the transfer of AFB1 from feeds into food of animal origin, especially milk, as AFM1.

Evaluating the actual exposure of livestock to AFs is not always simple, because these toxicants may not be uniformly distributed in feeds and grains. The occurrence of AFs in feeds is very important in on-farm feed storage, because certain temperature and humidity conditions promote the mould growth in isolated spots. In those circumstances, the presence of mould and their mycotoxins would be highly variable in a silo, with the absence of

mould and mycotoxins in most parts and very high values of AF concentration in few *hot spot* pockets.

From a practical standpoint, the use of highly contaminated feeds in dairy farms is unlikely. However, the occasional use of AFB1-contaminated feedstuffs may happen and this can lead to accidental milk contamination by AFM1, perhaps at concentrations above the tolerance levels. In cows (Trucksess *et al.*, 1983), sheep (Battacone *et al.*, 2003) and goats (Battacone *et al.*, 2012), the pattern of AFM1 concentration in milk subsequent to the intake of a single dose of AFB1 showed that AFM1 was first detected in milk in the first milking following the AFB1 intake. The highest AFM1

concentration was reached in the milk produced during the first 6-12 hours; thereafter, AFM1 concentration decreased rapidly and reached a contamination level under the EU limit (0.050 µg/kg) after 3-4 milkings (1.5-2 days). The time at which AFM1 was no longer detectable in milk was unaffected by the peak concentration of AFM1 in milk. These experimental results suggest that the occurrence of AFM1 in milk can be a transient and subtle risk, which might not be easily managed if feeds are not monitored appropriately.

For humans, the risk related to the consumption of AFM1-contaminated milk is related to the continuous ingestion of AFB1-contaminated feeds by lactating animals. For this

reason, the transfer of AFM1 into milk of cows (Veldman *et al.*, 1992; Diaz *et al.*, 2004; Masoero *et al.*, 2007), goats (Smith *et al.*, 1994; Rao and Chopra, 2001), and sheep (Battacone *et al.*, 2003, 2005 and 2009) fed contaminated diets during a medium-long period has been extensively investigated. Overall, several studies (Frobish *et al.*, 1986; Battacone *et al.*, 2009) showed that AFM1 excretion in milk was dose-related and reached a steady-state concentration after 2, 4 or more days of continuous ingestion of AFB1. The steady-state condition assumes that the AFB1 intake is in equilibrium with its excretion into milk as AFM1. This is the most appropriate condition to determine the carry-over rate, expressed as the ratio

**Table 1. Maximum levels of aflatoxin B1 in products intended for animal feed, as reported in Commission Regulation EC No 574/2011 (European Commission, 2011).**

Undesirable substance	Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feed with a moisture content of 12%
Aflatoxin B1	Feed materials	0.02
	Complementary and complete feed	0.01
	With the exception of: Compound feed for dairy cattle and calves, dairy sheep and lambs, dairy goats and kids, piglets and young poultry animals	0.005
	Compound feed for cattle (except dairy cattle and calves), sheep (except dairy sheep and lambs), goats (except dairy goats and kids), pigs (except piglets) and poultry (except young animals)	0.02

**Table 2. Guidance values for deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding, as reported in Commission Recommendation EC No 576/2006 (European Commission, 2006b).**

Mycotoxin	Products intended for animal feed ( )	Guidance value in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12 %
Deoxynivalenol	Feed materials	
	Cereals and cereal products with the exception of corn by-products	8
	Corn by-products	12
	Complementary and complete feedingstuffs with the exception of	5
	Those for pigs	0.9
Zearalenone	Those for calves (<4 months), lambs and kids	2
	Feed materials	
	Cereals and cereal products with the exception of corn by-products	2
	Corn by-products	3
Ochratoxin A	Complementary and complete feedingstuffs	
	For piglets and gilts (young sows)	0.1
	For sows and fattening pigs	0.25
	For calves, dairy cattle, sheep (including lamb) and goats (including kids)	0.5
Fumonisin B1+B2	Feed materials	
	Cereals and cereal products	0.25
	Complementary and complete feedingstuffs	
	For pigs	0.05
Fumonisin B1+B2	For poultry	0.1
	Feed materials	
	Corn and corn products	60
	Complementary and complete feedingstuffs for	
	Pigs, horses ( <i>Equidae</i> ), rabbits and pet animals	5
	Fish	10
	Poultry, calves (<4 months), lambs and kids	20
Adult ruminants (>4 months) and mink	50	

between the daily amount of AFM1 excreted into milk and the daily intake of AFB1. The extent of carry-over varies between animals and is mainly affected by dry matter intake and milk yield. In lactating cows, a positive relationship was observed between milk yield and carry-over rate of AFM1 (Veldman *et al.*, 1992; Masoero *et al.*, 2007). Milk components were not correlated with AFM1 in milk (Battacone *et al.*, 2012), suggesting that the passage of this toxin from the blood into the alveolar lumen in the mammary gland is due to a passive diffusion across the mammary gland epithelium (Gallo *et al.*, 2008). Furthermore, Masoero *et al.* (2007) reported that the total AFM1 excretion in cow's milk was not affected by high membrane permeability (high somatic cell count), as previously hypothesised by Veldman *et al.* (1992).

It would be very important to determine whether the current tolerance level for AFM1 in milk in the EU can be ensured by the current legislation for AFB1 in feed. Battacone *et al.* (2009) reported that in ewes fed diets with an AFB1 concentration similar to the EU maximum tolerated level (0.005 mg/kg), the AFM1 in milk exceeded approximately 1.5 times the EU maximum limit (0.050 µg/kg). Furthermore, lactating cows fed a TMR containing approximately 3.7 ppb of AFB1 yielded a milk exceeding 0.060 µg/kg AFM1 after 3 days of intake (Masoero *et al.*, 2007). These results indicate that the AFM1 levels found in milk might exceed the maximum acceptable level set by the EU, even when AFB1 in feeds comply with the current feed legislation.

Several studies reported the binding affinity of AFM1 with the protein fraction in milk, and consequently the enhancement of its concentration in cheese as compared to milk (Barbiroli *et al.*, 2007). Actually, a relevant portion of AFM1 remains in the whey, but this partitioning depends on the cheese-making procedure. In typical hard cheeses, such as Grana Padano and Parmigiano-Reggiano, AFM1 concentration was approximately 3 times higher in curd than in milk and this factor increased up to 4.5 in long maturing cheese (Manetta *et al.*, 2009). In soft cheese, like Crescenza, the AFM1 concentration was approximately 2.5 times higher in curd than in milk and approximately 50% of the toxin remained in the whey (Cattaneo *et al.*, 2008). Similar data were obtained in curd from sheep milk (Battacone *et al.*, 2005).

Poultry meat derived from animals fed diets contaminated with aflatoxins may pose a risk to consumers only when the AFB1 dietary levels are very high (Hussain *et al.*, 2010). No AFB1 or its metabolites were detected in the muscle of

broilers fed diets contaminated with AFB1 at 50 µg/kg (Bintvihok and Kositcharoenkul, 2006). In addition, the eggs of hens fed a diet supplemented with 2.5 mg/kg of AFB1 had no AFB1 or AFM1 residues (Zaghini *et al.*, 2005). Consequently, the AF risk for humans from consumption of meat products and eggs could be considered negligible.

### Prevention and management of mycotoxin-contamination in livestock production systems

Prevention of mould growth in feeds remains the best way to avoid or reduce mycotoxin intake in livestock. However, in some unusual field conditions, such as seasons characterised by warm temperatures and high humidity, the prevention efforts might be able to avoid mycotoxin contamination in agricultural commodities. In these cases, the use of detoxification techniques that are able to remove or reduce the effects of mycotoxins might be useful.

In recent years, the addition to feeds of substances that can suppress or reduce the bioavailability of mycotoxins in the gastrointestinal tract of animals has been evaluated. As a consequence, the EC (European Commission, 2009) defined a new group of feed additives as substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action. In reality, certain products allowed in the EU as technological feed additives are already added to feeds for their ability to sequester mycotoxins. With the entry into force of the Regulation No 1060/2013, the European Commission, based on EFSA's Opinions (European Food Safety Authority, 2011), authorised the use of bentonite as substances for the reduction of the contamination with AFB1 in feeds for ruminants, poultry and pigs (European Commission, 2013a). Moreover, a preparation of a micro-organism strain DSM 11798 of the Coriobacteriaceae family has been authorised as an additive in swine nutrition for its effect on the reduction of the contamination of feed by DON (European Commission, 2013b).

Generally, sequestering agents (SAs) can be considered effective in reducing the transfer of AFB1 from diets into milk as AFM1 in lactating animals or the negative effects due to mycotoxin ingestion in livestock. These compounds are able to bind several toxin molecules forming a stable complex in the gastro-intestinal tract (Jouany, 2007; Masoero *et al.*, 2009; Gallo and Masoero, 2010). The mechanisms involved

in the formation of the mycotoxin-SA complex are related to either weak chemical bonds, such as electronic elementary charges, hydrogen bonds, Van der Waals bonds, or physical capture (Phillips *et al.*, 1991; Diaz and Smith, 2005; Yiannikouris *et al.*, 2005; Phillips *et al.*, 2008). When added to animal diets, these compounds should be free of impurities, odourless and without any flavours (Diaz and Smith, 2005; Jouany, 2007), and low doses (*e.g.*, about 50-100 g per cow per day) are preferable to high doses to avoid a suspected, but not yet demonstrated, interaction between SAs and diet nutrients (European Food Safety Authority, 2009). In some cases, results reported in literature about the helpfulness of SAs in the livestock diet are not consistent, suggesting that the effectiveness of SA is affected by several factors, such as type and dose of the binder, level of feed contamination, duration of exposure to the toxin, and animal species and physiological condition (Kolossova and Stroka, 2011). Furthermore, different SAs did not show the same detoxicant activity for the different mycotoxins.

The main types of SAs currently employed on farm conditions belong to three main groups, namely clay minerals (*e.g.*, bentonite, hydrated sodium calcium aluminosilicate (HSCAS), zeolites, smectite, and montmorillonite; Phillips *et al.*, 1991; Ramos and Hernandez, 1996; Rao and Chopra, 2001; Diaz *et al.*, 2003; Jouany, 2007), yeast cell wall based products (Karaman *et al.*, 2005; Yiannikouris *et al.*, 2005) and activated carbons (Galvano *et al.*, 1996; Rao and Chopra, 2001; Diaz *et al.*, 2003; Diaz and Smith, 2005).

Before using SAs in animal diets, they should be experimentally tested to verify their capability for binding to mycotoxins and forming a stable mycotoxin-SA complex (Moschini *et al.*, 2008; European Food Safety Authority, 2009). Although *in vivo* studies could be the best experimental approach to investigate the efficiency of SAs in sequestering mycotoxins, they are expensive, time-consuming and their repeatability is often poor (Gallo *et al.*, 2014). Thus, *in vitro* approaches could be a suitable screening tool to verify the efficiency of SAs under controlled conditions, thus providing information on their mode of action (Lemke *et al.*, 2001; Avvantaggiato *et al.*, 2003; Moschini *et al.*, 2008). However, as stated in an EFSA Scientific Opinion (European Food Safety Authority, 2010), ... *in vitro* studies do not sufficiently mimic the conditions in the digestive tract, the differences between target animals and their metabolism, and consequently cannot be used to demonstrate efficacy under practical conditions. Taking this aspect into

account, several *in vitro* adsorption tests were proposed to screen the efficiency of SAs, differing mainly for the type of tested mycotoxin (pure or extracted from natural contaminated feeds), mycotoxin dilution factor (*w/v*), mycotoxin:SA ratio (*w/w*), pH conditions and media (water, buffer or biological fluid) in which the adsorption test was conducted (Ramos and Hernandez, 1996; Grant and Phillips, 1998; Ledoux and Rottinghaus, 1999; Gallo and Masoero, 2010). If a SA was considered efficient in sequestering mycotoxins (SA bind capacity higher than 80%) and forming a stable mycotoxin-SA complex (release of bounded mycotoxin lower than 5%) *in vitro*, then it could be tested *in vivo*. This aspect was stated also by EFSA experts (European Food Safety Authority, 2010) declaring that substances for reduction of the contamination of feed by mycotoxins have little or no effect in or on the feed itself until after ingestion by the animal. Consequently, some *in vivo* studies are required for their assessment.

Trials carried out on lactating dairy cows showed that an efficient SA was able to reduce by about 50% the excretion of AFB1 into milk as AFM1 (Masoero *et al.*, 2009; Pietri *et al.*, 2009). The sequestering activity of SAs could be improved by modifying the way in which they are included in the diet (Masoero *et al.*, 2009). For example, mixing a SA directly to the contaminated feed rather than to the whole diet could increase the efficiency of the SA by 20%. Furthermore, if the contact between the SA and the mycotoxin-contaminated feed occurred in the presence of water, such as during pelleting or extrusion, the sequestering efficiency could be increased up to 40% (Masoero *et al.*, 2009). For more insights concerning the efficiency of SAs for mycotoxins and their use in the diets of ruminants, pigs and poultry, readers can refer to an extensive review published by Kossolova *et al.* (2009).

### **Mycotoxin analysis in feedstuff as a tool to control and evaluate food safety**

The knowledge and control of the level and distribution of mycotoxins in food and feed is a topic of interest worldwide for consumers, producers, manufacturers, regulatory agencies and researchers, due to their high economic and sanitary impact on human and animal health. Because it is impossible to completely eliminate the presence of these contaminants, an adequate surveillance and frequent checks are fun-

damental to assure the quality and safety of raw materials destined for direct consumption by humans and animals or industrial processes.

The European Community fixed maximum levels for mycotoxins in food and feed through the Commission Regulation (EC) No 1881/2006, Directive 2002/32/EC, Commission Recommendation No 2013/165/EU, and Commission Recommendation No 2006/576 (Cheli *et al.*, 2013). The methods of sampling and analysis for the official control of the levels of mycotoxins in food and feed are covered by the Commission Regulation (EC) No 401/2006 and Commission Regulation (EC) No. 152/2009/EC (Cheli *et al.*, 2014). To guarantee food safety, the need for confirmatory methods of analysis with high sensitivity and accuracy, which meet the regulatory requirements, remains a critical issue. However, the traditional methods for the quantitative determination of mycotoxins in food and feed (mass spectrometric (MS), high-performance liquid chromatographic (HPLC) based on UV or MS detection) have some typical drawbacks, such as high costs of implementation, long time of analysis, low samples throughput, and need for highly qualified operator. Therefore, the availability of fast, reliable and simple methods to use as detecting tools for evaluating food and feed contaminants is one of the main challenges for a modern food and feed industry, in order to safeguard customer's health and improve production safety (Cheli *et al.*, 2012). In recent years, several cost-effective and suitable approaches have been proposed to assess the effectiveness of safety measures and to achieve logistical and operational goals. The use of fast analytical methods would save time, require less training and let products move rapidly through the industrial chain. Apart from the well-known and competitive enzyme linked immunosorbent assay (ELISA), several rapid methods have been considered for mycotoxin analysis, rapid screening and quantification, especially flow-through immunoassay, lateral flow device, fluorescence polarisation immunoassay capillary electrophoresis, surface plasmon resonance, and molecularly imprinted polymers. Although some of these methods were developed and validated for rapid and quantitative determination of some mycotoxins, they are destructive, and require an extraction step and, in some cases, a clean-up procedure. The application of analytical techniques, such as infrared spectroscopy (NIR, FR-NIR) and electronic nose, coupled with chemometric tools, is a rapid, non-invasive and promising analytical approach for mycotoxin analysis. Advantages and drawbacks of these technologies are reported in Table 3

(Cheli *et al.*, 2012). The development and implementation of fast, non-destructive, and applicable methods in a screening control procedure for the evaluation of the content of undesirable substances in food and feed must take into consideration the maximum levels or guidance values established by the EU. In particular, several authors highlighted the potential of the NIRS methodology as a fast and non-destructive tool for the detection of mycotoxins at contamination levels lower or close to the maximum permitted limit set by the EU (Pettersson and Aberg, 2003; De Girolamo *et al.*, 2009). Methods using the EN were developed by several authors for high throughput screening of DON contamination in durum wheat (Olsson *et al.*, 2002; Campagnoli *et al.*, 2011; Lippolis *et al.*, 2014), indicating that a robust and suitable electronic nose method is able to discriminate wheat samples at contamination levels close to the DON maximum permitted limit set by the EU.

In mycotoxin analysis, the analytical phase is the final phase of a complex sampling procedure. A sampling plan can be defined as a test procedure combined with specific analytical procedures and, in the case of undesirable substances, a sample acceptance limit (Cheli *et al.*, 2009). Because of the heterogeneous distribution of mycotoxins in food and feed, planning an effective sampling procedure for reliable mycotoxin detection or quantification is a major challenge for operators. All the phases, particularly the sampling technique, are associated with a high variability which can impair the reliability of the final result. Improper sampling invalidates all the analytical work, making it impossible to evaluate the effective degree or the risk of contamination, and sometimes generating unreasonable scare. Adequate sampling is necessary to make wise management decisions about what to do with lots of products that may be contaminated with mycotoxins (van Egmond *et al.*, 2007). Because sampling uncertainty likely leads to final uncertain results, even the choice of expensive, precise, sensible and specific analytical methods might result in an inefficient screening strategy. Instead, the adoption of a rapid, low cost and high-sample throughput analytical approach can be a better option. This is one of the most important reasons why research and development on these analytical approaches and specific statistical data analysis deserve further efforts. As stated by Fearn (2009), The safest policy is to use the simplest method you can, and within that the simplest model you can, avoiding the temptation to add a lot of extra complexity for a small gain in performance.



**Table 3. A list of the emerging rapid methods for mycotoxin analysis (modified from Cheli *et al.*, 2012).**

Methods	Advantages	Drawbacks	References
LFD	Rapid No expensive equipment Easy to use	Semi-quantitative Validation required for each matrix	Maragos (2004); Zheng <i>et al.</i> (2006); Goryacheva <i>et al.</i> (2007)
FPI	High sensitivity Low matrix interference	Not usable for simultaneous detection of several individual mycotoxins	Maragos (2004); Goryacheva <i>et al.</i> (2007)
CE	High sensitivity Non-polluting technology Possible simultaneous multi-component analysis	Expensive equipment Expensive Clean-up may be required	Maragos (2004); Maragos and Appel (2007)
SPR	Rapid No clean up	Cross reactivity	Tudos <i>et al.</i> (2003); van der Gaag <i>et al.</i> (2003); Maragos (2004)
MIP	Low cost Stable Reusable	Poor selectivity	Maragos (2004); Logrieco <i>et al.</i> (2005); Krska and Welzig (2006)
IR spectroscopy (NIR, FT-NIR)	Rapid Non destructive No clean up Easy to use	Expensive equipment Calibration model must be validated Good for classification	Kos <i>et al.</i> (2003); Pettersson and Aberg (2003); Berardo <i>et al.</i> (2005); De Girolamo <i>et al.</i> (2009)
EN	Rapid Non destructive No clean up	Calibration model must be validated Good for classification	Keshri and Magan (2000); Olsson <i>et al.</i> (2002); Presicce <i>et al.</i> (2006); Campagnoli <i>et al.</i> (2011); Lippolis <i>et al.</i> (2014)

LFD, lateral flow device; FPI, fluorescence polarization immunoassay; Ce, capillary electrophoresis; SPR, surface plasmon resonance; MIP, molecularly imprinted polymers; IR, infrared; NIR, near infrared; FT-NIR, Fourier-transform near infrared; EN, electronic nose.

## Conclusions

The risk analysis always is needed to comply with the EU rules aiming to control the food safety from the farm to the fork. In European Union a wide-ranging legislation is primed for the control of toxicant compounds in feed and food. When wide distribution of pollution sources occurs it may be useful: to monitor routinely the safety of animal products coming from rural areas subjected to potential pollution by different sources; to design and implement specific monitoring plans conceived to assess the quality and safety of animal products on an epidemiologic basis; to combine new technologies and official control plans to develop and apply new strategies aimed at the prevention of agro-environmental crisis and reduction of consumers' risk. Occurrence of mycotoxin contamination in feedstuffs have a relevant economic impact. Moreover, the hazard identification of mycotoxins in animal feeds and their transfer into food chain have to consider that: for some mycotoxins, like AFB1 and OTA, the transfer of toxicant along the food chain could be relevant in terms of food safety; compliance of mycotoxin contamina-

tion in feedstuffs is not always consistent with the guarantee of compliance for food; prevention of contamination is the most profitable and useful tool to ensure food safety; the addition of substances reducing the uptake of mycotoxin in the gastrointestinal tract of animals could represent a supplementary tool to improve safety guarantee in some adverse condition. Official methods for mycotoxins analysis, in feeds and foods, are available with performance properties meeting the general and specific requirements reported in EU Regulations. The development and establishment of fast, non-destructive, and actually applicable methods in a screening control procedure for the evaluation of undesirable substances content in food/feed must consider the maximum levels or guidance values established by the European Union. Since, in mycotoxin analysis, the sampling uncertainty dominates in the final uncertainty result, the choice of expensive, precise, sensible, specific analytical method could result an inefficient strategy. The adoption of a rapid, low cost but high sample throughput analytical approach able to test a high number of samples can represent a better option.

In conclusion, even if the complete absence of toxicants in food (zero risk) cannot current-

ly be assured, the present safety systems, for food of animal origin, have useful tools for monitoring all points along the food chain. However, implementations of control measures in various sectors of the food supply chain are necessary to ensure safe food. Those measures have to be directed towards monitoring programmes for environmental contaminants linked to industrialisation and urbanisation, pollutants, contaminants and natural toxins.

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