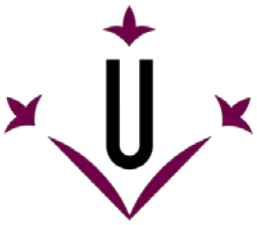

A review of the mycotoxin adsorbing agents, with an emphasis on their multi-binding capacity, for animal feed decontamination

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Abstract

Contamination of animal feed with mycotoxins still occurs very often, despite great efforts in preventing it. Animal feeds are contaminated, at low levels, with several mycotoxins, particularly with those produced by *Aspergillus* and *Fusarium* genera (Aflatoxin B_1, Ochratoxin A, Zearalenone, Deoxynivalenol and Fumonisina B_1). In animal feed, to date, only Aflatoxin B_1 is limited through EU regulation. Consequently, mycotoxins cause serious disorders and diseases in farm animals. In 2009, the European Union (386/2009/EC) approved the use of mycotoxin-detoxifying agents, as feed additives, to prevent mycotoxicoses in farm animals. The present review gives an overview of the problem of multi-mycotoxin contamination of feed, and aims to classify mycotoxin adsorbing agents (minerals, organic, and synthetic) for feed decontamination, focusing on adsorbents with the ability to bind to multiple mycotoxins, which should have a more effective application in farms but they are still little studied in scientific literature.

Keywords: mycotoxins, animal feed, adsorbents, multi-binding, decontamination

Abbreviations:

15-acetyldeoxynivalenol (15-Ac-DON), 3-acetyldeoxynivalenol (3-Ac-DON), Activated Carbon (AC), Adsorbing Agents (AA), Aflatoxin (AF), Aflatoxin B_1 (AFB1), Aflatoxin B_2 (AFB2), Aflatoxin M_1 (AFM1), Aflatoxins (AFs), Alternariol (AOH), Biotransforming Agents (BA), Body Weight (BW), Deoxynivalenol (DON), Ergot Alkaloids (EAs), Esterified Glucomannans (EGM), European Comission (EC), European Food Safety Authority (EFSA), European Union (EU), FAO (Food and Agriculture Organization of the United Nations), Feed Intake (FI), Fumonisin B_1 (FB1), Fumonisin B_2 (FB2), Fumonisins (FBs), Fusaric Acid (FA), Gastrointestinal (GI), Good Agricultural Practices (GAP), Glucomannans (GM), Hydrated Sodium Calcium Aluminosilicate (HSCAS), International Agency for Research on Cancer (IARC), Maximum Permitted Levels (MPLs), Molecular Weight (MW), Nivalenol (NIV), Ochratoxin A (OTA), Yeast Cell Wall (YCW), Weight Gain (WG), Zearalenone (ZEN).

Contents

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1. Introduction

1.1 Overview of mycotoxins in animal feed

Mycotoxins are low molecular weight (MW) secondary metabolites produced by filamentous fungi that have adverse effects at low levels on humans and animals. They have a significant impact on economies and international trade. Fungi producing mycotoxins are known as mycotoxigenic. Some of them are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungal species. The most relevant groups of mycotoxins found in animal feed are produced by three genera of fungi: *Aspergillus* (aflatoxins (AFs) and ochratoxin A (OTA)), *Penicillium* (OTA), and *Fusarium* species (trichothecenes, fumonisins (FBs), and zearalenone (ZEN)) (Fig. 1; Table 1) (Marin et al., 2013). They appear in the feed chain because of fungal infection of crops, and due to the use of mouldy grains and forage as components of animal feed. Fungi can invade and produce mycotoxins on the growing plants before harvesting (pre-harvest toxins), or produce toxins after harvest and during crop storage and transportation (postharvest toxins). In general, environmental conditions, such as high temperatures, high moisture levels, and insect damage, cause stress and predispose plants in the field to mould growth and mycotoxin contamination (Medina et al., 2015). Moreover, poor harvesting practices, improper drying, handling, packaging, and transport conditions contribute to increasing the risk of mycotoxin production (Bhat et al., 2010).

The economic consequences of mycotoxin contamination are very significant, and often crops with large amounts of mycotoxin have to be destroyed. The most susceptible crops to contamination with mycotoxins are cereals such as wheat, maize, barley, rye and oat (Cano-Sancho et al. 2010; Rodríguez-Carrasco et al., 2013; Vidal et al., 2013). Cereals constitute a major part of the daily diet of animals and they are important ingredients in animal compound feed (Pinotti et al., 2016). A high percentage of feed samples have been reported to be

contaminated with mycotoxins, and what is more, most of them have been reported to be contaminated with more than one mycotoxin (Kosicki et al., 2016; Streit el at., 2012; Zachariasova et al., 2014). In most cases, the concentrations were low enough to ensure compliance with the EU guidance values or Maximum Permitted Levels (MPLs) (Table 2, and 3). However, farm animals have shown to exhibit symptoms of chronic mycotoxicoses when exposed to feed contaminated with toxins below the guideline levels (Wielogórska et al., 2016). Additionally, farmers generally notice acute adverse effects on animal performance, such as low weight gain (WG), reproductive and metabolic disorders, with consequent economic losses, because not all mycotoxins found in animal feed have been regulated (see section 1.4). Therefore, producers often have to set internal limits more stringent than those regulated, in order to avoid losses.

Different strategies, including preventive measures at pre- and postharvest, have been developed to neutralize mycotoxins in animal feed such as good agricultural practices (GAP) and good storage practices (GSP). These actions are considered the best way of controlling mycotoxin contamination; however, even exercising of good practices might not completely avoid or eliminate mycotoxins in the feed chain (Di Gregorio et al., 2014). Moreover, the use of physical and chemical methods for the detoxification of agricultural commodities contaminated with mycotoxins is restricted due to the problems associated with safety issues, possible losses in nutritional quality coupled with limited efficacy and cost implications (EC, 2009; Kolosova and Stroka, 2011).

As mycotoxins cause serious diseases in farm animals, the EU approved the use of mycotoxin-detoxifying agents, by including a new group of feed additives defined as ‘substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action’ (EC, 2009). At the same time, the EFSA reported a review of mycotoxin-detoxifying agents used as feed additives (Boudergue et al., 2009) that covered

aspects such as mode of action, efficacy and feed/food safety. Since then, numerous studies have been published on the efficacy of the adsorbing agents (Di Gregorio et al., 2014; Magnoli et al., 2011; Neff et al., 2013; Nesic et al., 2008; Pfohl-Leszkowicz et al., 2015; Santos et al., 2011; Wang et al., 2012). However, most studies still focus on the efficacy of adsorption of a specific mycotoxin, usually tested at high levels which, as mentioned previously, in real settings is rarely the case.

In the light of the high co-occurrence of mycotoxins in agricultural commodities, the task of developing a more versatile solution for multi-toxin feed decontamination is challenging. The present review intends to address the problem of animal feed multi-mycotoxin contamination, as well as to classify mycotoxin adsorbing agents (minerals, organic and synthetic) for feed decontamination, focusing on adsorbents with the ability to bind to multiple mycotoxins, which are in great demand by animal feed producers, but are still little studied in scientific literature.

1.2 Occurrence and co-occurrence of mycotoxins in animal feed

Despite efforts to control fungal contamination, extensive mycotoxin contamination has been reported in both developing and developed countries. Recent surveys have been carried out to evaluate the worldwide incidence of mycotoxin contamination in feedstuffs and feed raw materials (Pinotti et al., 2016; Streit et al., 2013). On a global level, 30% to 100% of food and feed samples are contaminated (Pinotti et al., 2016). According to Streit et al. (2012) 72% of feed samples surveyed contained detectable levels of mycotoxins, while only 1-18% samples (depending on the toxin) presented levels above the EU guidelines or regulations. Also, Rodrigues and Naehrer (2012) studied the prevalence of mycotoxins in feedstuffs and finished feed worldwide between 2009 and 2010. Their results (from HPLC) revealed that 81% of the 6000 samples tested positive for at least one mycotoxin, although in many cases the regulatory and guidance levels were not surpassed. The most commonly

occurring mycotoxins were deoxynivalenol (DON) (65%), FBs (56%) and ZEN (44%), followed by AFs (31%) and OTA (27%). AF production occurred primarily in cereal samples from regions with tropical or subtropical climates such as Southern Europe, Africa, South and Southeast Asia. The amount of AFB1 was often the highest in the mixtures of AFs (Rodrigues and Naehrer, 2012). While DON contamination was observed worldwide, more than 60% of positive samples were found in samples (wheat, maize and barley) from North America, Northern and Central Europe, and North Asia (Rodrigues and Naehrer, 2012; Streit et al., 2012). The highest incidence of ZEN contamination (more than 30% of positive samples) was found in North and South America, Central Europe, Africa, and North and Southeast Asia (Rodrigues and Naehrer, 2012). On the other hand, FB contamination was found mostly in maize and maize products from ~~in~~ South America, Southern Europe, Africa, and Southeast Asia, FB1 being the most abundant (Rodrigues and Naehrer, 2012). Different *Fusarium* mycotoxins were often found jointly in contaminated cereals (Cano-Sancho et al., 2010; Stanciu et al., 2017). Lastly, OTA prevalence was highest in South Asia and Africa, but its distribution in contaminated feed lots tended to be very heterogeneous (Rodrigues and Naeher, 2012).

However, occurrence patterns of mycotoxins are changing as a consequence of rising average temperatures due to climate change (Medina et al., 2015; Miraglia et al., 2009; Wielogórska et al., 2016). In Southern Europe, AF contamination, previously uncommon in Europe, will become increasingly significant. In fact, Italian researchers detected AFB1 in cattle feed and AFM1 in cow's milk surpassing the maximum allowable in the EU, in 8.1% and in 1.7%, respectively (Decastelli et al., 2007).

Nevertheless, studying the occurrence of any given mycotoxin alone, provides incomplete information about the risk associated with the respective feedstuff considering the fact that mycotoxigenic fungi are usually capable of producing more than one mycotoxin, so animal

feed is particularly vulnerable to multiple contaminations (Streit et al., 2012). Results of feed surveys performed in Europe also highlight the problem of high levels of co-contamination with a number of different mycotoxins (Zachariasova et al., 2014). Of the 82% of feed samples that were contaminated, type B-trichothecenes and FBs occurred most often, 75% were co-contaminated with more than one mycotoxin while only two samples exceeded the recommended EU levels (Monbaliu et al., 2010). Along the same lines, Griessler et al. (2010) conducted a survey on feed and feed ingredients sourced in Southern Europe. The *Fusarium* mycotoxins (type B-trichothecenes, ZEN and FBs) were the major contaminants, while AFs and OTA were detected less frequently. It was further reported that 23% of all samples from Spain contained at least two mycotoxins.

Other researchers evaluated the level of mycotoxins in raw materials and products for animal nutrition in Poland in 2011-2014. A total of 1384 samples (maize samples, maize silage samples, small grain cereal samples and complete feed samples) were analysed for the occurrence of DON, Nivalenol (NIV), T-2 and HT-2 toxins, ZEN, FBs, OTA, and AFs. Also in this case, DON as well as ZEN were the most frequently occurring mycotoxins, present in 89% and 92% of maize samples, and in 86 and 88% of maize silage samples, respectively. Additionally, in 24 samples the content of mycotoxins exceeded EU recommendations. Regarding the complete feed, trichothecenes and ZEN were found in more than 90% of the samples (Kosicki et al., 2016).

Multi-mycotoxin contamination is a topic of great concern and seems to be increasing. The frequent detection of mycotoxin co-occurrence even in studies screening for a limited number of analytes underlines the importance of mycotoxin reduction strategies like the addition of multi-composition adsorbents in order to sequester a wider range of mycotoxins.

1.3 Toxic effects of major mycotoxins on farm animals

Animal feeds are routinely subject to contamination from diverse sources. AFB1, DON, ZEN, OTA and FB1 are considered the most economically significant mycotoxins in terms of their prevalence and their negative effects on animal performance (Table 1), (Di Gregorio et al., 2014). The diseases caused by short or long exposure to mycotoxins are known as mycotoxicoses. Clinical symptoms usually subside upon removal of contaminated feed. In general, smaller organisms are more susceptible to mycotoxin poisoning. Poultry, pigs, and also aquatic vertebrates are very sensitive to mycotoxins. Due to their high consumption of cereals, they are exposed to these toxins and to chronic contamination. Ruminants have, however, generally been more resistant to the adverse effects of mycotoxins, since the rumen microbiota is capable of degrading mycotoxins.

On the other hand, when mycotoxins are present simultaneously, interactive effects can be classified as additive, antagonistic or synergistic. Numerous reports of synergistic or additive effects mainly for AFs in combination with FBs, trichothecenes, OTA or mixtures of various *Fusarium* toxins have been published (Ruiz et al., 2011). The studies show that co-contaminated samples may exhibit adverse health effects even with concentrations of toxins being within regulatory limits (Grenier and Oswald, 2011).

The economic impact of mycotoxins includes loss of human and animal life, increased health care and veterinary care costs, and reduced livestock production (Zain, 2011).

Aflatoxins

AFs are relatively hydrophilic molecules produced by fungi of the genus *Aspergillus*. AFB1 is the most potent naturally occurring carcinogen classified by the IARC as group 1 (IARC, 1993, 2002). It is the only mycotoxin with an established MPL in feedstuffs (see section 1.4 and Table 2). Lactating animals fed AFB1 contaminated feeds will produce milk contaminated with its monohydroxylated derivative AFM1, classified by the IARC as group 2B, possibly carcinogenic to humans (IARC, 1993, 2002). Chronic toxicity is the most

common form of aflatoxicosis and it is caused by the consumption of relatively small amounts of these toxic compounds over an extended period. The main target organ of AFB1 toxicity is the liver, where it may be metabolized into different metabolites (Di Gregorio et al., 2014). The effects of long term exposure of levels AFs are associated with reduction in WG, decreased milk or egg production, increased disease susceptibility, reduced feed efficiency, tumours and teratogenicity (Table 1) (Streit et al., 2012). Poultry, cattle, and swine are the domestic species of greatest economic concern in terms of aflatoxicosis. Some species of fish and birds are extremely sensitive to the toxic and carcinogenic action of AFB1 (Anater et al., 2016). In poultry, dietary 2.5 mg AFs/kg significantly reduced the feed intake (FI) by 9-11% (Rawal et al., 2010). Also reductions of WG of 30% in chickens following the consumption of feed contaminated at levels of 0.03 mg AFB1/kg of feed, were observed (Boudergue et al., 2009). In ruminants, first effects were observed at levels of 1-2 mg AFB1/kg of feed such as lower feed ingestion and milk yield in cattle, while in pigs some mortalities and liver diseases were observed within one month after ingestion of contaminated feeds at 0.8-3 mg AFB1/kg (Meissonnier et al., 2005). In fish (*Oreochromis niloticus*, Nile tilapia) a reduction in growth rate was observed along with hepatic damage at levels from 0.245 mg AFB1/kg of feed (Matejova et al., 2017). Rainbow trout is considered the most sensitive fish species to AFs (Anater et al., 2016).

Ochratoxin A

OTA is a nephrotoxic mycotoxin that causes renal toxicity and possesses carcinogenic, teratogenic, immunotoxic and possibly neurotoxic properties. This toxin has been classified by the IARC as a possible human carcinogen (Group 2B) (IARC, 2002). In animals, it has been shown that after a prolonged OTA intake, a nephropathy linked to the degeneration of the convoluted tubule of the nephron and renal interstitial fibrosis occurs, followed by a decrease in the thickness of the basal membrane and glomerular hyalinization (Pfohl-

Leszkowicz et al., 2015). Ruminants seem to be resistant to OTA exposure. In pigs, acute ochratoxicosis episodes mention kidney diseases (nephropathy), while in chronic ochratoxicosis the first signs were reductions of feed consumption and WG at the level of 1-1.4 mg OTA/kg of feed (Boudergue et al., 2009). In poultry, nephropathy was reported to occur from level of 2 mg OTA/kg of feed, and the first signs of chronic ochratoxicosis were noticed at a minimal level of 0.5 mg OTA/kg of feed in laying hens and chickens (Boudergue et al., 2009). Also, in catfish adverse effects were observed at concentration of 1.0 mg OTA/kg feed (Matejova et al., 2017).

Deoxynivalenol

DON belongs to the trichothecene B group and, although being one of its least acutely toxic members, is of particular interest owing to its high prevalence in cereals worldwide. DON is also known as vomitoxin, and it is primarily known for causing feed refusal, weight loss, decreases nutritional efficiency and causes lesions in the GI tract, vomiting, bloody diarrhoea and severe dermatitis accompanied by haemorrhaging (Bryden, 2012). Other symptoms are immune disorders, such as immunosuppression or immunostimulation. In pigs, the most sensitive of the susceptible species, DON has also a neurotoxic effect that produces an anorexic syndrome, by altering the concentration of neurotransmitters in the hypothalamus, cerebellum, and frontal cortex (Marin et al., 2013). DON may be produced together with two acetylated derivatives, 3-acetyldeoxynivalenol (3-AcDON) and 15-acetyldeoxynivalenol (15-AcDON), which have differential toxicity on pig intestine (Pinton et al., 2012). The first signs of reduction of feed consumption in pigs, were observed at levels of 1-3 mg of DON/kg feed (Marin et al., 2013). Regarding ruminants, the animal species least sensitive to DON, feed refusal syndrome was noticed in cows after consumption during 10 weeks of a wheat concentrate level of 6.4 mg DON/kg feed (Boudergue et al., 2009). In poultry, which seem to be relatively resistant to DON compared to other livestock, the effects vary according to the

species. Yunus et al. (2010) found that dietary levels of DON below and above the recommended limits (1.8 and 18 mg/kg, respectively) affected chicken performance and organ status to various degrees, while according to Awad et al. (2006), concentrations above 5 mg DON/kg of diet are necessary to cause detrimental effects in poultry. Trout are extremely sensitive to DON. A significant decrease in FI, WG, growth rate, and feed efficiency in trout exposed to diets naturally contaminated from 0.3 to 2.6 mg DON/kg of feed for 8 week was observed (Anater et al., 2016).

Zearalenone

ZEN is a non-steroidal estrogenic toxin produced by certain *Fusarium* species. ZEN, and some of its metabolites, can competitively bind to oestrogen receptors leading to reproductive disorders and estrogenic dysfunction in humans and animals (especially in breeding animals), impairing fertility and increasing the frequency of stillbirths along with reducing sperm quality (Zinedine et al., 2007). During pregnancy, ZEN reduces embryo survival and foetal weight. Additionally, ZEN produces vulvar dilatation and redness, retention or absence of milk, and rectal prolapse (Zinedine et al., 2007). Swine are also the animal species most severely affected by ZEN. In pigs, first signs of estrogenic syndrome appeared from 3-7 days on a ZEN contaminated diet at levels of 1.5 mg/kg feed (Boudergue et al., 2009). Regarding ruminants, a reduction of fertility in dairy cattle was observed for levels of ZEN above 0.5 mg/kg feed (Boudergue et al., 2009). Among the livestock species of interest, poultry seems to be the most resistant to ZEN. Based on experimental studies, levels above 100 mg ZEN/kg feed are needed to see the first signs of intoxication (Boudergue et al., 2009). Otherwise, a slight tendency toward prolonged clotting time and lowered iron concentrations in the liver and ovary after exposing rainbow trout to 10 mg ZEN/kg for 24, 72, and 168 h was observed by Wozny et al. (2012).

Fumonisins

Acute and chronic toxicity by FBs has been largely demonstrated in several animal species, including carcinogenicity and cardiovascular toxic effects (Voss et al., 2007). Based on toxicological evidence, the IARC has classified FB1 as possibly carcinogenic to humans (group 2B) (IARC, 2002). The liver and kidney are the major target organs for FB toxicity (Voss et al., 2007), but also the intestine is a possible target (Bouhet and Oswald, 2007). In horses, consumption of FB contaminated feeds has been recognized as a cause of an illness known as equine leukoencephalomalacia (ELEM). The first symptoms are lethargy, blindness, and decreased feed intake, followed by convulsions and death after several hours or days (Morgavi and Riley, 2007). Similarly, FB1 contaminated feeds have shown to cause a syndrome known as porcine pulmonary edema (PPE) in pigs (Table 1). Clinical signs usually include decreased feed consumption, dyspnoea, weakness, cyanosis, and death (Morgavi and Riley, 2007). Based on several studies, levels above 100 mg FB1/kg feed are needed to get first signs of zootechnical disturbance in pigs (Boudergue et al., 2009). Cows and poultry are considerably less sensitive to FBs than horses and pigs (Boudergue et al., 2009).

On the other hand, in fish, FBs have a disruptive effect on neural and liver tissues. Sensitivity to FB1 in fish is dependent on both species and individual body weight (BW) (Anater et al., 2016).

1.4 European regulation of mycotoxins in feedingstuffs

Approximately 100 countries have developed specific limits for mycotoxins, while the number of regulated mycotoxins differs for food and feed, for example between the US and the EU. According to FAO, Europe has the most extensive regulations for mycotoxins in feed. Canadian regulations are among the most detailed, as they additionally include mycotoxins not regulated in EU feedstuffs such as diacetoxyscirpenol, DAS (a type A trichothecene), with China and Iran also having demanding limits in place (FAO, 2004). Nevertheless, regulations in the rest of the world undoubtedly focus mainly on AFs, with only 15 countries in Africa

having specific, feed oriented mycotoxin regulations in place (FAO, 2004). All countries with mycotoxin regulations have at least regulatory limits for AFB1 or the sum of aflatoxins B1, B2, G1, and G2 in foods and/or feeds (Wielogórska et al., 2016).

Currently in the EU, AFB1 is the only mycotoxin with MPLs set in feeds under Directive 2002/32. For animal feed, MPLs set by the EC for AFB1 is 0.02 mg/kg for all feed materials (EC, 2003). The current limits for AFB1 in animal feedingstuffs range from 0.005 to 0.02 mg/kg (Table 2), (Directive 2003/100/EC). Additionally, guidance values have been recommended for a further five mycotoxins in feedstuffs: DON, ZEN, OTA and FBs (2006/576/EU) (Table 3). Recently the EC has established recommendations on the presence of T-2 and HT-2 toxin in cereals and cereal products for feed and compound feed (2013/165/EU) (Table 3). Also, a maximum level of 500 mg/kg of rye ergot (*Claviceps purpurea*) sclerotia has been set for unprocessed cereals in order to avoid the presence of ergot alkaloids in food and feed (2015/1040/EU). However, food/feed may be contaminated with a much wider range of mycotoxins. In fact, the EFSA has recently issued scientific opinions on the risk to human and animal health related to the reported presence of *Alternaria* toxins (EFSA, 2011).

On the other hand, European regulations for animal-derived products have been imposed, especially for AFM1 in milk (0.025-0.05 µg/kg) (EC No 1881/2006), which in particular imply a strict control of the AFB1 content in feed for dairy cattle. Otherwise, the EC has not established MPLs of OTA in meat or other animal products. However, some countries have enforced MPLs of OTA concentrations, for example Denmark (pig kidney 10 µg/kg), Estonia (pig liver 10 µg/kg), Romania (pig kidney, liver and meat 5 µg/kg), Slovakia (meat 5 µg/kg, milk 5 µg/kg). Others countries have developed national guidelines for recommended maximum OTA levels, for example Italy (pig meat and derived products 1 µg/kg) (FAO, 2004).

2. Prevention and decontamination of mycotoxins in food and feed

There are multiple possible origins of fungal infection, so prevention strategies for fungal and mycotoxin contamination must be carried out at an integrative level all along the food production chain (plant growth, harvest, storage and distribution). The intervention should occur before any fungal infestation, or during the period of mould invasion of plant material and mycotoxin production, and also when the agricultural products have been identified as heavily contaminated (Jouany, 2007). Several codes of practices have been developed by Codex Alimentarius for the prevention and reduction of mycotoxins in cereals, and raw materials. These recommendations are divided into two parts: recommended practices based on GAP and Good Manufacturing Practices (GMP), and the use of Hazard Analysis and Critical Control Points (HACCP) (Awad et al., 2010; FAO, 2001).

Different pre- and postharvest control strategies have been extensively reviewed elsewhere (Awad et al., 2010; Jouany, 2007; Kabak et al., 2006). Common practical measures include planting of more resistant varieties of cereals, selection of high quality seeds, avoiding high plant densities, preventive management towards insect infestations as well as suitable management of crop residues that are often the primary inoculum of mycotoxigenic fungi. Appropriate field management practices such as crop rotation, soil cultivation, irrigation, and fertilisation are known to influence mycotoxin formation in the field (Awad et al., 2010; Kabak et al., 2006). Prediction models integrating some of these field parameters and weather input are being developed to assess the risk of mycotoxin contamination of pre-harvest cereals (Jouany, 2007). Careful selection of harvest date, equipment and harvesting procedures to minimise crop damage and removal of damaged crops and high moisture plant parts also reduces mould infections. During postharvest, storage and distribution, the control of moisture levels of stored grains (less than 15%), maintenance at low temperatures as well

as preservation of the integrity of grains are critical in preventing mycotoxin production (Kabak et al., 2006). The development of resistant hybrids appears to be a very promising technology, but commercial hybrids are not always available (Abbas et al., 2009). On the other hand, cereals modified by genetic engineering could be used to limit the risk of fungal infection. Commercially, this technology is based on the use of plants with resistance to insect attack, which indirectly cause a reduction in fungal infection and mycotoxin contamination. Genetic research is also being carried out on the induction of mycotoxin detoxification pathways or inhibition of mycotoxin production in the grain (Duvick, 2001; Karlovsky, 2011). However, in Europe genetically modified food is still not well received by the population.

When prevention is not achieved at field level or during harvest, decontamination procedures such as physical treatments of contaminated grains can be used including washing, polishing, mechanical sorting and separation, density segregation, and flotation. However, the efficiency of these techniques depends on the level of contamination and the distribution of mycotoxins throughout the grain. Additionally, the results obtained are uncertain and often connected with high product losses.

With regard to chemical decontamination methods, they require not only suitable reaction facilities but also additional treatments (drying, cleaning) that can make them time consuming and expensive (Jouany, 2007). Nevertheless, various chemicals including oxidising and reducing agents, acids, bases, salts and chlorinating substances have been tested for their ability to degrade mycotoxins in agricultural commodities. Only a limited number of these are effective without diminishing the feed nutritional value or palatability (Kolosova and Stroka, 2011). Chemically, some mycotoxins can be destroyed with calcium hydroxide, monoethylamine, ozone or ammonia. Particularly, ammoniation is an approved procedure for

the detoxication of AF contaminated feed in several countries (Boudergue et al., 2009). However, the use of chemical decontamination processes is not legal within the EU (Directive 2002/32). With regard to mycotoxin decontamination, the EC is in favour of the use of physical decontamination processes such as the use of adsorbents (see section 3.1) and sorting procedures (Directive 2002/32).

3. Detoxifying agents

Although prevention of mycotoxin contamination in the field and during storage is the main goal of agricultural and feed industries, the absence of mycotoxins in the ration of farm animals cannot be fully assured. Due to the increasing number of reports on the presence of mycotoxins in feeds, there is a rise in demand for practical decontamination procedures. It should be pointed out that the mixing of batches with the aim of decreasing the level of contamination below the maximum tolerable level is not permitted under Directive 2002/32.

In 2009, a new functional group was added in the category of technological feed additives. This group is defined by the Commission Regulation (EC) No 386/2009 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*' (EC, 2009). These substances are known as detoxifying agents. The use of such products does not mean that animal feed exceeding the established MPLs may be used. The additives are added to the diet of animals (mainly of swine, poultry and cattle) in order to reduce the absorption of mycotoxins from the GI tract and their distribution to blood and target organs. Depending on their mode of action, they act either by binding mycotoxins to their surface (adsorption), or by degrading or transforming them into less toxic metabolites (biotransformation). Therefore, we can define at least two main categories: adsorbing agents and biotransforming agents.

- **Adsorbing agents (AA)**

Mycotoxin-AA are large MW compounds which bind the mycotoxins present in contaminated feed without dissociating in the GI tract of the animal, thus limiting their bioavailability after ingestion, decreasing exposure of animals to mycotoxins. Mycotoxins may bind to AA by different types of interactions such as hydrophobic binding, hydrogen bonds, electrostatic attraction or repulsion and coordination bonds (Di Gregorio et al., 2014). In this way the mycotoxin-AA complex passes through the animal and is eliminated via the faeces. This complex has to be stable throughout the entire digestive track, so its stability in varying pH, which is influenced by the AA physical properties (total charge and charge distribution, the size of the pores, and the accessible surface area) and targeted toxins' physicochemical properties (polarity, solubility, and shape), is one of the crucial parameters to be evaluated in order to prevent desorption of the toxin (Avantaggiato et al., 2005; Huwig et al., 2001; Kabak et al., 2006). AA are also known as mycotoxin binders, mycotoxin binding agents, sequestering agents, or adsorbents. These agents can be divided in three sub-groups: inorganic compounds, organic or synthetic (Di Gregorio et al., 2014; Jard et al., 2011).

- **Biotransforming agents (BA)**

Another strategy is the degradation of mycotoxins into non-toxic metabolites by using BA. Biotransformation can be achieved by mycotoxin-degrading enzymes or by microorganisms producing such enzymes. Several microbial species, including bacteria, yeast and fungi have been recognised for their ability to biotransform mycotoxins into less toxic metabolites through routes such as (de)acetylation, oxygenation, ring/side chain cleavage, deepoxidation, isomerisation or glucosylation (Wielogórska et al., 2016). *Eubacterium* BBSH 797 strain isolated from bovine rumen fluids was one of the most studied species being able to efficiently degrade DON, and other trichothecenes, which after *in vitro* and *in vivo* tests was

introduced onto the market as the commercial biotransforming product Mycofix® BBSH (Biomin, Getzersdorf, Austria). Also, some of the enzymes responsible for biotransforming characteristics recognized in these microbial species, have been isolated and applied directly as detoxifying agents (Boudergue et al., 2009). However, the application in practice of BA is limited due to lack of information about transformation mechanisms, the toxicity of products derived from biotransformation, the effect of transformation reactions on the nutritional values of feeds, and safety towards animals (Wielogórska et al., 2016).

3.1 Adsorbing agents (AA)

3.1.1 Inorganic adsorbents

Aluminosilicates

Aluminosilicates constitute the most abundant group of rock-forming minerals. The basic structural unit of silicate clay minerals consists of the combination of silica tetrahedral and aluminium octahedral sheets, both with oxygen and hydroxyl groups (Di Gregorio et al., 2014). Most studies on the alleviation of mycotoxicosis by the use of AA have focused on aluminosilicates. Within this group, there are two major subclasses: phyllosilicates and tectosilicates. Phyllosilicates include bentonites, montmorillonites, smectites, kaolinites, and illites. They can adsorb substances on their surface or within their interlaminar space. The tectosilicates include zeolites. They provide a large and specific binding surface but also size, shape and charge selectivity due to which they have been compared to molecular sieves (Huwig et al., 2001). Inactivation of mycotoxins by adsorbents has been reviewed by many authors (Avantaggiato et al., 2007, 2005, 2003; Diaz and Smith, 2005; Jouany, 2007; Huwig et al., 2001; Kabak et al., 2006; Kong et al., 2014; Phillips et al., 2008; Ramos at al., 1996a; Vekiru et al., 2007; Vila-Donat et al., 2017a,b). However, most of the AA appear to bind to only a limited group of mycotoxins while showing very little or no binding to others (Jouany, 2007; Kabak et al., 2006; Kong et al., 2014; Ramos and Hernández, 1997; Ramos et al.,

1996a; Vekiru et al., 2007; Vila-Donat et al. 2017a,b). Furthermore, it should be noted that clays could adsorb micronutrients and have negative effects on the bioavailability of minerals and trace elements (Kolosva and Stroka, 2011). Also, the risk of natural clays to be contaminated with dioxins and metals has to be considered (Jouany, 2007).

- Hydrated sodium calcium aluminosilicate (HSCAS)

HSCAS (calcium montmorillonite clay) are commonly used in animal feed as anti-caking agents. HSCAS has shown to act as an enterosorbent that tightly and selectively binds AFs in the GI tract of animals decreasing their bioavailability and associated toxicity (Harper et al., 2010; Neff et al., 2013; Phillips et al., 2008). Evidence suggests that AFs may react at multiple sites on HSCAS particles, especially the interlayer region, but also at edges and basal surfaces (Kolosova and Stroka, 2011). Other mechanisms of AFB1 sorption by HSCAS surfaces may involve the chelation or interaction of AFB1 with interlayer cations (especially Ca) or various edge-site metals (Di Gregorio et al., 2014). HSCAS is quite effective with respect to AFs but fails to prevent toxic effects of *Fusarium* mycotoxins, such as FBs, or trichothecenes (Avantaggiato et al., 2005; Harper et al., 2010; Kabak et al., 2006; Neff et al., 2013; Phillips et al., 2008; Ramos and Hernández, 1997).

- Bentonites (montmorillonites)

Bentonites are phyllosilicate clays with a layered crystalline microstructure of variable composition. They are frequently referred as smectites because it is the dominant mineral clay. Smectite includes mainly montmorillonite. The adsorption effectiveness of bentonite depends on the montmorillonite content and the interchangeable cations (Kolosova and Stroka, 2011). Montmorillonite is composed of layers of octahedral aluminium and tetrahedral silicon coordinated with oxygen atoms. The large surface area and high cation exchange capacity of the smectite group make them capable of adsorbing organic substances

by the penetration of both cations and polar molecules. Bentonites have demonstrated a large efficacy on mycotoxins adsorption, specifically AFs (Kong et al., 2014; Magnoli et al., 2011; Ramos and Hernández, 1996; Thieu et al., 2008; Vekiru et al., 2007; Vila-Donat et al., 2017a,b), and other mycotoxins (ZEN, OTA and FBs) in numerous *in vitro* and *in vivo* studies (Avantaggiato et al., 2005; Miazzo et al., 2005; Ramos et al., 1996a,b; Wang et al., 2012). According to Deng et al. (2010), under dry conditions AF molecules bind to smectite by direct ion-dipole interactions and by coordination between exchangeable cations and carbonyl groups, whereas under humid conditions, AF molecules bind to smectite by H-bonding between the carbonyl oxygens and hydration-shell water (the authors provide a graphical interpretation of the interaction between AF and smectite).

The safety and efficacy of bentonite as feed additive has also been evaluated by the EFSA. It has been observed that bentonites are not genotoxic and are not absorbed following application as a feed additive, hence providing no direct toxicological risk for the animal (EFSA, 2011). A bentonite/dioctahedral montmorillonite (Mycofix®, Biomin, Austria) has been the first-ever product authorized by the EU as AA with proven mycotoxin counteracting properties that fulfils the strict requirements on AF-binding capability according to EC 1060/2013.

- Zeolites

The zeolite structure consists of an assemblage of SiO_4 and AlO_4 tetrahedra joined together in various regular arrangements through shared oxygen atoms to form an infinite three-dimensional cage-like structure. The partial substitution of Si^{4+} by Al^{3+} results in an excess of negative charge which is compensated by alkali and earth alkaline cations such as sodium, calcium and potassium ions (Dakovic et al., 2003; Huwig et al., 2001). Zeolites have a large internal surface, associated with its elevated cation exchange capacity and with the

adsorption of polar molecules (Di Gregorio et al., 2014). Some studies have shown that natural zeolite-clinoptilolite have the ability to adsorb AFs and other mycotoxins such as FBs (Dakovic et al., 2010). However, modified zeolites are more effective than natural ones towards FBs (Baglieri et al., 2013) (see section 3.1.3). The role of zeolites as feed additives on the prevention of certain farm animal diseases has been also reviewed by Papaioannou et al. (2005).

Other clays:

Other mineral adsorbents such as diatomite (a mineral formed by the accumulation and fossilisation of diatomaceous algae shells in lacustrine and marine environments) and sepiolite (a complex magnesium silicate belonging to the group of hormites) have been studied as mycotoxin adsorbents over the last few years. The negative charges associated with the high specific surface area make sepiolite an adequate sorbent for some polar molecules. Sepiolite showed good results in the adsorption of AFs, however the efficiency of adsorption of other mycotoxins is limited. Usually sepiolite is associated with bentonite and both have similar properties such as high surface area and significant sorption capacity. However, the cation exchange capacity of sepiolite is much lower than that of smectite (Di Gregorio et al., 2014). Regarding diatomaceous earth, it is fossilized by the deposit of silica in its structure, which is arranged in thin or thick layers, interspersed by clay lenses. This material is very thin with a porous structure, high surface area and microstructure mainly composed of amorphous silica, or opal and frustules. Diatomaceous earth is used as anti-caking agent during feed processing (Weaver et al., 2013).

3.1.2 Organic adsorbents

Facing the relative inefficacy of the clay adsorbents towards mycotoxins other than AFs, natural organic binders have been proposed (Avantaggiato et al., 2014; Jouany, 2007; Mezes

et al., 2010; Ringot et al., 2007). The mixture of inorganic and organic adsorbents could make them more adapted to the most frequent cases of multi-contaminated feeds.

- Yeast cell wall (YCW)

Saccharomyces cerevisiae occurs as part of natural microbial populations in foods and it is used as a starter culture in fermented food and beverages. YCW mainly consists of proteins, lipids and polysaccharides, with glucans and mannans being the two main constituents of the latter fraction. In fact, YCW exhibits a great variety of accessible mycotoxin adsorption loci as well as different binding mechanisms (hydrogen bonds, ionic or hydrophobic interactions) (Ringot el al., 2007). Adsorption on the cell wall surface is an interaction between the toxins and functional groups of the cell surface. YCW has shown much larger sorption capabilities across a wider spectrum of mycotoxins such as ZEN, OTA, and FBs (Fruhauf et al., 2012; Pfohl-Leszkowicz, et al., 2015; Shetty and Jespersen, 2006), including DON, being the β-D-glucan fraction of YCW directly correlated with the binding process (Faucet-Marquis et al., 2014). Also mannans (from *S.cerevisiae*) have demonstrated to be effective at binding DON at different pH values, with adsorption rate decreasing as DON concentration increases (Cravet et al., 2010). Furthermore, esterified glucomannans (EGM) have been proved to be effective in counteracting the toxic effects of different mycotoxins simultaneously exposed (Aravind et al., 2003; Avantaggiato et al., 2005; Li et al., 2012; Mohaghegh et al., 2017).

- Lactic acid bacteria (LAB)

LAB are a group of gram-positive, acid-tolerant, generally non-sporulating bacteria that have common metabolic and physiological characteristics. These bacteria, usually found in decomposing plants and dairy products, produce lactic acid as the major metabolic end-product of carbohydrate fermentation. Some LAB strains *(Lactobacillus rhamnosus*) displayed the ability to bind certain compounds (e.g. AFB1 and ZEN) in the small intestine

with cell wall peptidoglycans, polysaccharides and teichoic acid proposed as crucial elements in that process. Gram-positive bacteria seem more efficient towards non-polar toxins (such as ZEN) due to higher hydrophobicity of the cell surface (Kabak et al., 2006). The strength of the mycotoxin-LAB interaction seems to be influenced by the peptidoglycan structure and, more precisely, by its amino acid composition (Dalié et al., 2010). Moreover *L. rhamnosus* is considered a safe and effective chemopreventive because of its use in various dairy products including yogurt.

- Micronized fibres and bio-sorbents

Micronized fibres can be obtained from different plant materials such as cereals or legumes (wheat, barley, alfafa, oat, pea hulls). They consist mainly of cellulose, hemicellulose and lignin, and they have been utilised as mycotoxin adsorbents due to favourable gut adsorption and enhanced faecal excretion (Aoudia et al., 2009). Particularly, micronized wheat fibres exhibited beneficial effects against OTA adsorption (Aoudia et al., 2009). However, the binding ability for AFB1 of the cellulose products is less compared with the values of other inorganic adsorbents (Kong et al., 2014).

Regarding bio-sorbents, red wine waste such as dehydrated grape pomace (rich in phenolic compounds) has recently been demonstrated *in vitro* to be an excellent adsorbent for simultaneously removing several mycotoxins in a liquid medium (AFB1, ZEN, OTA and FBs) (Avantaggiato et al., 2014). In addition, apple pomace (rich in fibres and pectin) was tested previously in pigs as mycotoxin adsorbent by incorporating it in DON-contaminated feed, and authors suggested that the negative effect of DON may be attenuated (Gutzwiller et al., 2007). Furthermore, interesting findings were obtained from *in vitro* studies regarding humic acids, originating from natural decaying of organic plant materials. They also have shown the capacity to adsorb mycotoxins, especially AFB1, OTA and ZEN (Sabater-Vilar et al., 2007; Santos et al., 2011).

- Activated carbon (AC)

AC is a non-soluble powder, produced by pyrolysis of several organic compounds, followed by its chemical or physical activation aimed at developing a highly porous structure. Based on literature data, AC seems to be the most effective adsorbent with high affinity for different mycotoxins (including DON) *in vitro* (Avantaggiato et al., 2005, 2004, 2003; Diaz and Smith, 2005; Mezes et al., 2010; Ramos et al., 1996a,b; Sabater-Vilar et al., 2007). Nevertheless the *in vitro* efficacy of AC toward some mycotoxins was not confirmed *in vivo* (Avantaggiato et al., 2005). Generally, the adsorption properties of AC depend on the source materials, surface area and pore size distribution (Kolosova and Stroka, 2011). However, AC is unspecific, hence essential nutrients are also adsorbed particularly if their concentrations in feed are much higher compared to those of a mycotoxin (Van Alfen, 2014). Vekiru et al. (2007) also reported that AC strongly adsorbed vitamins and minerals essential for growth and development. Moreover, when they were analysed *in vivo*, components of the food matrix can compete or inhibit the interaction with a mycotoxin (Wielosgorksa et al., 2016).

It should be noted that organic adsorbents, especially cereal or leguminous fibres as well as pulp and peels of fruits, may contain fungal contamination, so they would have to be analysed before use to rule out the presence of mycotoxins.

3.1.3 Synthetics

- Organoaluminosilicates or modified clays

In animals, aluminosilicates appear to be selective in their 'chemisorption' of AFs with little or no beneficial effect against ZEN, OTA and FBs. This limitation can be overcome by chemical modifications. These consist of alterations of surface properties by exchange of structural charge-balance cations with high molecular weight quaternary amines, which results in an increased hydrophobicity (Papaioannou et al., 2005). *In vitro* results have

verified the binding efficacy of modified montmorillonite and clinoptilolite against ZEN and OTA (Dakovic et al., 2003; Jiang et al., 2012; Papaioannou et al., 2005). Moreover, other authors have shown organically modified clays are more effective than natural clays towards FBs (Baglieri et al., 2013; Dakovic et al., 2010; Doll et al., 2005). Specifically, Baglieri et al. (2013) showed that the addition of modified clays (2%) to contaminated maize allowed a reduction of more than 70% of the amount of FB1 released in solution. Also, *in vitro* adsorption of ZEN by a modified montmorillonite nanocomposite was reported by Feng et al. (2008). This material demonstrated the ability to bind ZEN in aqueous solutions with little nonspecific adsorption of common nutrients, such as vitamins. Moreover it showed very low desorption rate and higher adsorption capacity of ZEN compared to unmodified montmorillonite nanocomposite. Nano grade particle size and hydrophobic properties of modified montmorillonite nanocomposite seems responsible for the specific adsorption (Feng et al., 2008).

- Polymers

Polymers, such as cholestyramine (an anion exchange resin), divinylbenzene-styrene and polyvinylpyrrolidone (a highly polar amphoteric polymer) have been demonstrated to bind mycotoxins *in vitro* and *in vivo* (Avantaggiato et al., 2005; Jard et al., 2011; Jouany, 2007; Mezes et al., 2010; Ramos et al., 1996b). Cholestyramine is an insoluble quaternary ammonium exchange resin which strongly binds various anionic compounds and may weakly adsorb neutral or cationic compounds by non-specific binding. This compound proved *in vitro* to be an effective adsorbent for OTA, FBs and ZEN (Avantaggiato et al., 2005, 2003; Döll et al., 2004; Ramos et al., 1996b). Its efficacy (inclusion up 2% in feed) was confirmed by GI models (ZEN) and by *in vivo* experiments (FBs) (Avantaggiato et al., 2003, 2005; Kolosova and Stroka, 2011). Also, cholestyramine reduced DON levels in buffer (pH 7) by

approximately 60% (Cavret et al., 2010), while only 5% of DON could be adsorbed by cholestyramine in a dynamic digestive tract (Avantaggiato et al., 2005).

However, the high cost of these polymers would be a limiting factor for practical applications.

4. Adsorbing agents with multi-toxin efficacy

4.1 *In vitro* studies

In binding studies *in vitro*, the efficacy of the AA seems to be mainly dependant on the physicochemical properties of the adsorbent and mycotoxin, as well as the pH. Also, binding efficiency of the adsorbent is highly affected by the presence of the matrix or even gastric juice which can decrease its performance compared to the results obtained in buffer (Jaynes et al., 2007; Vekiru et al., 2007). Other factors such as feed structure, moisture content and oxygen availability during testing could heavily affect the results of binding studies (Paulick et al., 2015). Moreover, they appear to be highly influenced by the levels of mycotoxins themselves, their co-occurrence or possible co-operative effects (Faucet-Marquis et al., 2014; Santos et al., 2011).

To date, most of the *in vitro* binding studies in scientific literature has focused on the efficacy of adsorption of a specific mycotoxin, usually tested at high levels. Moreover, most of them have been carried out using buffer solutions, and in many cases without feed matrix. However in the “real-world”, animal feed is rarely contaminated by a single mycotoxin, and metabolic digestions are not taken into account on *in vitro* testing, unless GI models or biological juices have been used. As a result, there are many discrepancies between *in vitro* and *in vivo* studies. Accordingly, only *in vitro* studies focused on the adsorption of multiple mycotoxins, and carried out taking into account the aforementioned points, have been highlighted in this section.

Following this line, Avantaggiato et al. (2004) investigated for the first time the intestinal absorption of two mycotoxins (DON and NIV) simultaneously. Before this, an *in vitro* screening test of 14 adsorbents (Mycorsorb®, Mycofix Plus®, Myco AD®, cholestyramine, bentonite, zeolite, AC, glucomannans (GM), etc.) was carried out. Successively, the efficacy of AC (the only one that showed reductions) in adsorbing DON and NIV by using a dynamic GI model was tested. The *in vitro* intestinal absorptions were 51% and 21% respectively, of the 170 µg and 230 µg ingested through contaminated spiked wheat. The use of a GI model simulating animal conditions confirmed that DON absorption mainly occurred at the small intestinal level and specifically the jejunum.

Also, Santos et al. (2011) evaluated two different adsorbents (organic activated bentonite and humic acid) intended for use as feed additives in the prevention or reduction of the adverse effects exerted by 0.1 mg OTA/kg and 0.5 mg ZEN/kg in a common *in vitro* model, with a pH course comparing pH that can be expected in the digestive system of a monogastric animal (pH 7.4, pH 3.0, and pH 8.4). Both AA showed an adsorbing capacity of > 96% towards both mycotoxins, regardless pH, except for the humic acid product, that showed extensive desorption at pH 8.4.

Later, Avantaggiato et al. (2007) investigated combined mycotoxin binding properties of different compounds to enhance multi-binding capacity. They performed an *in vitro* study to assess the multi-toxin-binding efficacy of a carbon/aluminosilicate-based product (a mixture of six components, “Standard Q/FIS”) in a 2 % concentration, by using a dynamic GI model. On the basis of the results, a reduction of mycotoxin absorption was observed (up to 88% for AFB1, 44% for ZEN, 29% for FB1 and OTA), suggesting that this multi-composed adsorbent could be beneficial in reducing both individual and combined adverse effects of mycotoxins in animals. Most recently, the same research team examined the ability of an organic adsorbent, grape pomace (rich in phenolic antioxidants), to simultaneously adsorb

different mycotoxins from solution. Results showed that AFB1 was the most (83%) adsorbed mycotoxin followed by ZEN (67%), OTA (62%), and FB1 (29%), whereas the adsorption of DON was negligible. This study proved for the first time that grape pomace can simultaneously adsorb several mycotoxins, and that mycotoxins did not compete for adsorption in a multi-mycotoxin system. Hydrophobic interactions may be associated with AFB1 and ZEN adsorption, whereas polar non covalent interactions may be associated with OTA and FB1 adsorption. So grape pomace may have a wide range of technological applications as bio-sorbent to decontaminate multi-mycotoxin contaminated feed (Avantaggiato et al., 2014).

In vitro studies have to be confirmed *in vivo*. Thus, studies with animals would be necessary to prove the effectiveness of these adsorbents in reducing the toxic effects of mycotoxins without affecting the regular utilization of essential nutrients in animal feed, such as vitamins and minerals.

4.2 *In vivo* studies

Co-contamination is more likely to occur in the field than mono-contamination by mycotoxins (Grenier and Oswald, 2011; Streit et al., 2012; Zachariosova et al., 2014). The toxicity and clinical signs observed in animals when feed is multi-contaminated by mycotoxins are complex and diverse. The effects of specific mycotoxins and adsorbents differ for each animal species (Magnoli et al., 2011). Consequently, the evaluation of the efficacy of the AA against the different mycotoxins present in feeds is undertaken separately for poultry, swine, ruminants, and other species. Nevertheless, variability exists in the literature even within the same species. These variations may arise due to differences in adsorbents and doses, mycotoxin levels, animals' age, nutritional, health status, BW, as well as the sensitivity of the animals exposed (Neeff et al., 2013).

In this section, different *in vivo* trials, mainly with poultry and swine, testing the efficacy of the adsorbents in counteracting mycotoxin effects produced as a consequence of multi-toxin contaminated FI, have been summarized below and on Table 4. Data on multi-mycotoxin trials with other animal species are scarce in scientific literature.

Broilers:

With regard to studies with poultry, Miazzo et al. (2005) investigated the use of sodium bentonite (0.3%) to detoxify diets containing a combination of two mycotoxins (2.5 mg AFB1/kg + 200 mg FB1/kg) in broiler chicks. In broilers fed with co-contaminated feed (AFB1+FB1), the relative weights of organs (liver, kidney, gizzard and spleen) increased. Particularly, livers of birds were enlarged, yellowish, friable, and had rounded borders. Liver histopathology showed multifocal and varied cytoplasmatic vacuolization. The addition of bentonite to the contaminated diet counteracted these effects by reducing the incidence and severity of the pathological changes. The authors concluded that sodium bentonite was effective to counteract only some of the AFB1 promoted effects. It could be attributed to competition between AFB1 and FB1 for the active surfaces sites of the adsorbent, rendering a greater bioavailability of AFB1 in the presence of high dose of FB1.

Some authors pointed out that combined use of different adsorbents with diverse structural properties would provide versatile tools for preventing multi-mycotoxicosis by binding a wider range of mycotoxins (Avantaggiato et al., 2007; Huwig, 2001). On these lines, Liu et al. (2011) performed experiments *in vivo* on the effects of different mycotoxin adsorbents including EGM (0.05%), HSCAS (0.2%) and a combination of both (EGM + HSCAS, 0.1%) on performance, nutrient retention and meat quality in broilers fed on mould-contaminated maize (0.45 mg AFB1/kg, 0.06 mg OTA/kg, and 0.32 mg T-2/kg). The results indicated that mycotoxins in contaminated feed retard growth, nutrient retention and meat

quality, whereas the addition of HSCAS helped correct the decreased FI and the retention of phosphorus. The addition of EGM decreased yellowness in breast muscle, and the combination of both adsorbents (EGM + HSCAS, 0.1 %) significantly improved BW gain and FI. Moreover, the retention of crude lipid, crude protein, ash and phosphorus increased with the mix, making it the most effective treatment. The authors stressed that the addition of a combination of adsorbents to contaminated feed would be a versatile way of preventing mycotoxicosis.

More recently, a Greek research group examined *in vivo* the use of two bentonites (1%) differing in composition, as potential binders of three mycotoxins present in broiler diets at levels not exceeding the EU maximum (0.013 mg AFB1/kg, 0.1 mg OTA/kg and 1 mg ZEN/kg). The study revealed that the examined bentonites may maintain optimum broiler performance when mycotoxins present in the feed do not exceed EU limits and guidance values, possibly by ameliorating the negative effects of other mycotoxins, not regulated by EU, present at the same time in the feed since multi-contamination is more likely to occur in the field than single contamination (Pappas et al. 2014).

With regard to organic sorbents, Aravind et al. (2003) assessed the ability of EGM (0.05%) to alleviate the adverse effects of several combinations of mycotoxins (0.168 mg AFB1/kg, 0.0084 mg OTA/kg, 0.053 mg ZEN/kg, 0.032 mg T-2/kg) naturally found in broiler feed (Table 4). The naturally contaminated diet significantly decreased BW, feed consumption and feed efficiency, and increased the relative weights of liver and gizzard. Moreover, it was associated with significant decreases in urea nitrogen and haematocrit values along with altered γ-glutamyl transferase activity. However, the addition of EGM to a contaminated diet increased performance and serum levels of urea nitrogen and decreased the activity of γ-glutamyl transferase. These results suggested that EGM at 0.05% counteracted

the toxic effects of the naturally multi-contaminated diet alleviating growth depression and reducing organ weights.

Other researchers studied the effects of EGM as an adsorbent in feed multi-contaminated with DON and ZEN (Girish et al., 2008; Swamy et al., 2002; Yegani et al., 2006). Swamy et al. (2002) studied the effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins (9.7 mg DON/kg, 21.6 mg FA/kg, 0.8 mg ZEN/kg) on production parameters, clinical chemistry, and muscle coloration in broilers, and the possibility of EGM (0.2%) in counteracting these adverse effects. The feeding of contaminated grains caused significant linear increases in blood erythrocyte count and serum uric acid concentration and a significant linear decline in the serum lipase activity. EGM supplementation did not have any effect on feed consumption, WG or feed efficiency but counteracted most of the blood parameter alterations caused by the *Fusarium* mycotoxin-contaminated grains and reduced breast muscle redness (Swamy et al., 2002). Similar results were reported by Girish et al. (2008) who described the efficacy of GM included in naturally *Fusarium* contaminated feeds by preventing some blood and immunological parameter alterations on turkeys, and also by Yegani et al. (2006) on broiler breeder hens (Table 4).

Li et al. (2012) also investigated the toxicity of feed-borne *Fusarium* mycotoxins (102.08 mg AF/kg, 281.92 mg ZEN/kg, 5,874.38 mg FB/kg, 2,038.96 mg DON/kg) on physiological, biochemical and immunological parameters of broiler chickens and evaluated the efficacy of YCW (2%) adsorbent in preventing adverse mycotoxin-induced effects. They demonstrated that naturally contaminated diets induced adverse changes in organ weight, serum biochemistry, and immunological parameters as compared with broiler chickens fed the control diet. However, the addition of YCW (2%) adsorbent to contaminated feeds showed a positive protective effect on the relative weight of the liver, spleen, bursa of Fabricius and thymus, antibody titers of Newcastle disease, and splenic mRNA expression of IL-1β, IL-6,

and IFN-γ. The addition of YCW thus neutralized the detrimental effects of the naturally contaminated feed.

Very recently, Mohaghegh et al. (2017) evaluated the effect of Mycosorb® (EGM 0.05-0.1%) on performance, immunity, blood haematological and serum biochemical parameters in broilers exposed to diets naturally contaminated with mycotoxins (0.4273 mg AFB1/kg, 0.20631 mg DON/kg, and 0.0022 mg OTA/kg), (Table 4). Mycotoxin contamination affected chicken performance, organ weights, some haematological and most of the serum biochemical parameters. Dietary EGM supplementation (0.5, 0.1%) considerably improved the decreased BW gain and FI. However, only EGM (0.1%) ameliorated the negative impact of mycotoxins on the feed conversion ratio. Results indicated that supplementing EGM, particularly at 0.1% level, efficiently reversed the adverse effects of mycotoxins on broiler chickens.

Swine:

In pigs, the presence of trichothecenes (mainly DON) in feed is usually concomitant with ZEN (Gutzwiller et al., 2007; Swamy et al., 2003; Weaver et al., 2014). Doll et al., (2005) investigated the effects of dietary inclusion of a modified aluminosilicate (0.4%) in maize contaminated with *Fusarium* toxins (8.6 mg DON/kg and 1.2 mg ZEN/kg) for piglets. They could not demonstrate any detoxifying capacity in the tested additive. However, when much lower dosages were tested (0.14-0.31 mg DON/kg and 0.16-1.55 mg ZEN/kg) in sows, a beneficial effect of zeolite (clinoptilolite) was observed on the reproductive performance of the animals, since it resulted in larger litter sizes and piglets' BW at both birth and weaning (Kyriakys et al., 2002).

Recently, Weaver et al. (2013) determined the ability of three different feed additives (A: calcium montmorillonite clay, 2%; B: sodium bentonite, sepiolite clay and a dried brewer's

yeast component, 1.5%; C: a mixture of diatomaceous earth and yeast culture, 1.1%) to ameliorate the chronic negative effects of feeding diets containing 0.15 mg AFB1/kg and 1.1 mg DON/kg to pigs. The additives A and B in co-contaminated diets, reduced mycotoxin effects on the immune system and the liver, and showed some ability to improve growth. The additive C played a role in reducing liver damage. In general, the authors conclude that AFB1 and DON can be harmful to the growth and health of pigs consuming mycotoxins chronically. The selected feed additives improved pig health. However, the ability of feed additives to reduce mycotoxin was variable, and their function may depended on other factors, such as mycotoxin type, contamination level and pig health.

Regarding organic adsorbents, the efficacy of a polymeric GM in preventing *Fusarium* mycotoxicosis in pigs fed with a blend of naturally contaminated grains (5.5 mg DON/kg, 20.9 mg FA/kg, 0.4 mg ZEN/kg and 0.3 mg 15-Ac-DON/kg) was also tested (Table 4). Supplementation of GM polymer (0.2%) prevented some toxin-induced changes in metabolism. This may have been because this material is a high-MW polymer and acted by adsorbing mycotoxin molecules in the intestinal lumen and prevented uptake into blood and target tissues; however, GM polymer did not prevent the mycotoxin-induced growth depression (Swamy et al., 2003). More recently, Weaver et al. (2014) determined the effects of feeding naturally contaminated corn (4.8 mg/kg DON and 0.3 mg/kg ZEN) on pig performance and health status, and the ability of two yeast based feed additives (YCW and yeast fermentation product) to help pigs to manage the problem of mycotoxins. Results of this study indicated that the consumption of the contaminated feed reduced FI and BW gains, and increased oxidative DNA damage. However, the yeast fermentation additive improved the growth performance of pigs by increasing FI and WG, while the YCW product did not significantly improve the growth performance, but tended to reduce oxidative stress

associated with the consumption of mycotoxins (Weaber et al., 2014). Thus, the addition of both, the YCW product and yeast fermentation product, showed some benefits in reducing the effects of mycotoxins on pigs. However, the authors underlined these responses may vary under different mycotoxin concentrations, types, and mixtures.

On the other hand, Gutzwiller et al. (2007) studied the effects of *Fusarium* toxins (2.1-3.2 mg DON/kg 0.06-0.25 mg ZEN/kg) on growth, humoral immune response and internal organs in weaner pigs, and the efficacy of apple pomace (8%) in alleviating the negative effects induced by these mycotoxins. Results showed that pomace may alleviate the negative effect of DON on growth but does not counteract the hormonal effects of ZEN (Table 4).

Veal:

Regarding veal, only one study was found in literature. Specifically, Martin et al. (2010) studied the effects of feeding veal calves with corn naturally contaminated with the *Fusarium* mycotoxin (10.27 mg DON/kg, 1.27 mg 15-Ac-DON/kg and 1.84 mg ZEN/kg) and evaluated the modified YCW (1%) on performance, immunity and carcass characteristics of calves. Veal calves were able to tolerate a moderate feeding level of corn grains naturally contaminated with *Fusarium* mycotoxins. As there were generally no negative effects of the mycotoxin level in the diet, the efficacy of YCW as a mycotoxin adsorbent could not be assessed, but YCW alone had negligible effects on performance. The authors underlined that the reaction of calves depends on the compounds, concentration, duration of exposure and combinations of different mycotoxins in the diet.

5. Conclusions

A very high percentage of cereal-based animal feed is contaminated with more than one mycotoxin, the major contaminants being *Fusarium* mycotoxins (DON, ZEN and FBs). Only a low percentage of feed samples is contaminated above permitted/guideline levels. However,

animals (poultry, fish, and pigs, particularly) exhibit symptoms of mycotoxicosis even when exposed to feed contaminated with mycotoxins below the guidance levels, probably as a consequence of negative synergistic effects produced when different mycotoxins are simultaneously present in feed.

The use of many of the available physical and chemical methods for the decontamination of agricultural commodities contaminated with mycotoxins is restricted due to the problems associated with safety issues, possible losses in the nutritional quality of treated commodities coupled with limited efficacy and cost implications. The use of the adsorbents as feed additives is the only practical solution to feed decontamination, although it should be adapted to the current demands of farmers and AA have to be widely studied to guarantee their effectiveness and safety.

Based on the available data, inorganic adsorbents such as aluminosilicates (bentonites, HSCAS and some zeolites) have extensively demonstrated efficacy towards AFs. However, their efficacy against other mycotoxins, such as trichothecenes, is limited. Only bentonite and zeolite seem capable of partially adsorbing ZEN. On the other hand, modified clays are proving to be quite more effective than other unmodified clays in adsorbing FBs, OTA and ZEN. In addition, it has been showed that organic adsorbents (YCW, EGMs, dietary fibres or bio-sorbents) are more effective in binding to a wider spectrum of mycotoxins (ZEN, OTA, FBs).

Regarding DON, only some YCW (β-glucans and mannans), AC or synthetic polymers (cholestyramine) have been identified as potential adsorbents. However, a mix of different AA could give a cumulative efficacy or synergy due to their specific characteristics. On the other hand, combining mycotoxin binding properties of different adsorbents (mineral and organic), could be further adapted to the most frequent cases of multi-contaminated feed.

Most of the *in vitro* studies published in scientific literature are focused on adsorbent efficacy toward a single mycotoxin (very often AFB1, since it is the only one with MPLs in animal feed). Moreover, many assays have been performed with buffer solutions, and by testing with toxins and adsorbents far above regulated levels. Few *in vitro* binding studies have been performed by using GI models (with physiological juices at different pH) and focused on multi-mycotoxin adsorption. The latter, conducted recently, cover interactions thus closing the gap between *in vitro* and *in vivo* testing, and could reduce the number of animal studies. However *in vitro* predictions about the ability of adsorbents to protect animals from the adverse effects of mycotoxins should be approached with caution and should be confirmed *in vivo* with animals.

Regarding multi-binding *in vivo* studies, there are many studies with chickens and even with pigs, but other animal species such as cows and fish have been studied very little or not at all, probably because of the long duration and the high costs of experiments. In addition, the concentrations of adsorbent and mycotoxin used in the reviewed works are highly variable. Available data indicate that observed effects depend on the level and type of mycotoxin, as well as duration of exposure, type and dose of adsorbent, on the animal species and physiological condition of the animal. However, the combination of different adsorbents (mineral and organic) seems to be more effective in better counteracting the adverse effects produced by the simultaneous exposure of several mycotoxins in feed. In any case, future *in vivo* studies should be done by assaying naturally multi-contaminated feed, using "real-world" mycotoxins and adsorbent concentrations, tested at levels within the EU-regulations, and taking into account EFSA report endpoints; paying attention to their efficacy and safety, and their potential for interactions with critical nutrients (vitamins and minerals).

Animal feed is increasingly made from plant materials such as cereals and cereal by-products, and multiple contamination is increasingly observed in these raw materials. So a

potential adsorbent would have to be efficient in dealing with several mycotoxins. The development of such products would be an interesting trend in trying to counteract the toxic effects of co-occurring mycotoxins in animal feed. Therefore, further research should be conducted towards the achievement of this goal.

Declaration of interest statement

The authors declare there are no conflicts of interest

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References

Abbas, H.K., Wilkinson, J.R., Zablotowicz, R.M., Accinelli, C., Abel, C.A., Bruns, H.A. Weaver, M.A. 2009. Ecology of *Aspergillus flavus*, regulation of aflatoxin production, and management strategies to reduce aflatoxin contamination of corn. *Toxin Rev*. 28, 142-153.

Anater, A., Manyes, L., Meca, G., Ferrer, E., Bittencourt, F., Turra, C., Font, G. 2016. Mycotoxins and their consequences in aquaculture. A Review. *Aquaculture*. 451, 1-10.

Aoudia, N., Callu, P., Grosjean, F., Larondelle, Y. 2009. Effectiveness of mycotoxin sequestration activity of micronized wheat fibres on distribution of ochratoxin A in plasma, liver and kidney of piglets fed a naturally contaminated diet. *Food Chem. Toxicol*. 47, 1485-1489.

Aravind, K.L, Patil, V.S, Devegowda, G., Umakantha, B., Ganpule, S.P. 2003. Efficacy of Esterified Glucomannan to Counteract Mycotoxicosis in Naturally Contaminated Feed on Performance and Serum Biochemical and Hematological Parameters in Broilers. *Poult. Sci.* 82, 571-576.

Avantaggiato, G., Greco, D., Damascelli, A., Solfrizzo, M., Visconti, A. 2014. Assessment of multi-mycotoxin adsorption efficacy of grape pomace. *J. Agric. Food Chem*. 62, 497-507.

Avantaggiato, G., Havenaar, R., Visconti, A. 2003. Assessing the zearalenone-binding activity of adsorbent materials during passage through a dynamic *in vitro* gastrointestinal model. *Food Chem. Toxicol*. 41, 1283-1290.

Avantaggiato, G., Havenaar, R., Visconti, A. 2004. Evaluation of the intestinal absorption of deoxynivalenol and nivalenol by an *in vitro* gastrointestinal model, and the binding efficacy of activated carbon and other adsorbent materials. *Food Chem. Toxicol*. 42, 817-824.

Avantaggiato, G., Havenaar, R., Visconti, A. 2007. Assessment of the multi-mycotoxin-binding efficacy of a carbon/aluminosilicate-based product in an *in vitro* gastrointestinal model. *J. Agric. Food Chem*. 55, 4810-4819.

Avantaggiato, G., Solfrizzo, M., Visconti, A. 2005. Recent advances on the use of adsorbent materials for detoxification of *Fusarium* mycotoxins. *Food Addit. Contam*. 22, 379-388.

Awad, W.A., Böhm, J., Razzazi-Fazeli, E., Zentek, J. 2006. Effects of feeding deoxynivalenol contaminated wheat on growth performance, organ weights and histological parameters of the intestine of broiler chickens. *J. Anim. Physiol. Anim. Nutr*. 90, 32-37.

Awad, W.A., Ghareeb, K., Böhm, J., Zentek, J. 2010. Decontamination and detoxification strategies for the *Fusarium* mycotoxin deoxynivalenol in animal feed and the effectiveness of microbial biodegradation. *Food Addit. Contam.* 27, 510-520.

Baglieri, A., Reyneri, A., Gennari, M., Negre, M. 2013. Organically modified clays as binders of fumonisins in feedstocks. *J. Environ. Sci. Health* 48, 776-783.

Bhat, R., Rai, R.V., Karim, A.A. 2010. Mycotoxins in Food and Feed: Present Status and Future Concerns. *Compr. Rev. Food Sci. Food Saf.* 9, 57-81.

Boudergue, C., Burel, C., Dragacci, S., Favrot, M.C., Fremy, J.M., Massimi, C., Prigent, P., Debongnie, P., Pussemier, L., Boudra, H., Morgavi, D., Oswald, I., Perez, A., Avantaggiato, G. 2009. Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety. External Scientific Report Submitted to EFSA. Available on: https://www.efsa.europa.eu/en/supporting/pub/en-22 (Accessed on December 2017).

Bouhet, S., Oswald, I.P. 2007. The intestine as a possible target for fumonisin toxicity. *Mol. Nutr. Food Res*. 51, 925-931.

Bryden W.L. 2012. Mycotoxin contamination of the feed supply chain: implications for animal productivity and feed security. *Anim. Feed Sci. Technol*. 173, 134-158.

Cano-Sancho, G., Marin, S., Ramos, A.J., Sanchis, V. 2010. Biomonitoring of *Fusarium* spp. mycotoxins: perspectives for an individual exposure assessment tool. *Food Sci. Technol*. 16, 266-276.

Cavret, S., Laurent, N., Videmann, B., Mazallon, M., Lecoeur, S. 2010. Assessment of deoxynivalenol (DON) adsorbents and characterisation of their efficacy using complementary *in vitro* tests. *Food Addit. Contam.* 27, 43-53.

Dakovic, A., Kragovic, M., Rottinghaus, G.E., Sekulic, Z., Milicevic, S., Milonjic, S.K., Zaric, S. 2010. Influence of natural zeolitic tuff and organozeolites surface charge on sorption of ionizable fumonisin B1. *Colloids Surf. B*. 76, 272-278.

Dakovic, A., Tomasevic-Canovic, M., Rottinghaus, G., Dondur, V., Masic, Z. 2003. Adsorption of ochratoxin A on octadecyldimethyl benzyl ammonium exchanged-clinoptilolite-heulandite tuff. *Colloids Surf. B*. 30, 157-165.

Dalié D.K.D., Deschamps A.M., Richard-Forget, F. 2010. Lactic acid bacteria - Potential for control of mould growth and mycotoxins: A review. *Food Control*, 21, 370-380.

Decastelli, L., Lai, J., Gramaglia, M., Monaco, A., Nachtmann, C., Oldano, F., Ruffier, M., Sezian, A., Bandirola, C. 2007. Aflatoxins occurrence in milk and feed in Northern Italy during 2004-2005. *Food Control*, 18, 1263-1266.

Deng, Y. J., Velázquez, A. L. B., Billes, F., Dixon, J.B. 2010. Bonding mechanisms between aflatoxin B_1 and smectite. *Appl. Clay. Sci*. 50, 92-98.

Di Gregorio, M.C., de Neeff, D.V., Jager, A.V., Corassin, C.H., de Pinho Carão, A.C., de Albuquerque, R., de Azevedo, A.C., Fernandes Oliveira, C.A. 2014. Mineral adsorbent for prevention mycotoxins in animal feed. *Toxin Rev*. 33, 125-135.

Diaz, D.E., Smith, T.K. 2005. Mycotoxin sequestering agents: Practical tools for the neutralisation of mycotoxins. The Mycotoxin Blue Book. Nottingham, United Kingdom: Nottingham University Press; pp. 323-339.

Doll, S., Danicke, S., Valenta, H., Flachowsky, G. 2004. *In vitro* studies on the evaluation of mycotoxin detoxifying agents for their efficacy on deoxynivalenol and zearalenone. *Arch. Anim. Nutr.* 58, 311-324.

Doll, S., Gericke, S., Danicke, S., Raila, J., Ueberschar, K.H., Valenta, H., Schnurrbusch, U., Schweigert, F.J., Flachowsky, G. 2005. The efficacy of a modified aluminosilicate as a detoxifying agent in *Fusarium* toxin contaminated maize containing diets for piglets. *J Anim. Physiol. Anim. Nutr*. 89, 342-358.

Duvick, J. 2001. Prospects for reducing fumonisin contamination of maize through genetic modification. *Environ. Health Persp.* 109, 337-342.

EC, 2015. European Commission. Comission Regulation EU 2015/1940 of 29 October 2015 concerning maximum levels of ergot sclerotia in certain unprocessed cereals and the provisions on monitoring and reporting. *Off. J. EU.* L 283, 3-6.

EC, 2003. European Commission. Commission Directive 2003/100/ EC of 31 October 2003 amending Annex I to Directive 2002/32/ EC of the European Parliament and of the Council on undesirable substances in animal feed. *Off. J. EU.* L 285, 33-37.

EC, 2006. European Commission. Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. *Off. J. EU.* L 229, 7-9.

EC, 2009. European Commission. Commission regulation (EC) No. 386/2009 of 12 May 2009 amending Regulation (EC) No. 1831/2003 of the European Parliament and of the Council as regards the establishment of a new functional group of feed additives. *Off. J. EU*. L 118, 66.

EC, 2012. European Commission. Commission Recommendation of 15 March 2012 on the monitoring of the presence of ergot alkaloids in feed and food. *Off. J. EU*. L 77, 20-21.

EC, 2013a. European Commission. Commission Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products. *Off. J. EU.* L 91, 12-15.

EC, 2013b. European Commission. Commission Regulation (EU) No 1060/2013 of 29 October 2013 concerning the authorisation of bentonite as feed additive for all animal species. *Off. J. EU.* L 289, 33-37.

EFSA, 2011. European Food Safety Authority. Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. *EFSA J*. 9, 2407.

Faucet-Marquis, V., Joannis-Cassan, C., Hadjeba-Medjdoub, K., Ballet, N., Pfohl-Leszkowicz, A. 2014. Development of an *in vitro* method for the prediction of mycotoxin binding on yeast-based products: case of aflatoxin B1, zearalenone and ochratoxin A. *Appl. Microbiol. Biotechnol.* 98, 7583-7596.

Feng, J.L., Shan, M., Du, H.H., Han, X.Y., Xu, Z.R. 2008. *In vitro* adsorption of zearalenone by cetyltrimethyl ammonium bromide-modified montmorillonite nanocomposites. *Micropor. Mesopor. Mat.* 113, 99-105.

FAO, 2001. Food and Agriculture Organization of the United Nations. Manual on the application of the HACCP system in mycotoxin prevention and control. FAO Food and Nutrition Paper 73. FAO, Rome, Italy. Available at: http://www.fao.org/3/a-y1390e.pdf (Accessed on December 2017).

FAO, 2004. Food and Agriculture Organization of the United Nations. Worldwide regulations for mycotoxins in food and feed. Available at: http://www.fao.org/tempref/docrep/fao/007/y5499e/y5499e00.pdf (Accessed on December 2017).

Fruhauf, S., Schwartz, H., Ottner, F., Krska, R., Vekiru, E. 2012. Yeast cell based feed additives: studies on aflatoxin B1 and zearalenone. *Food Addit. Contam.* 29, 217-231.

Girish, C.K., Smith, T.K., Boermans, H.J., Karrow, N.A. 2008. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on performance, hematology, metabolism, and immunocompetence of turkeys. *Poult. Sci.* 87, 421-432.

Grenier, B., Oswald, I.P. 2011. Mycotoxin co-contamination of food and feed: meta-analysis of publications describing toxicological interactions. *World Mycotoxin J.* 4, 285-313.

Griessler, K., Rodrigues, I., Handl, J., Hofstetter, U. 2010. Occurrence of mycotoxins in Southern Europe. *World Mycotoxin J. 3*, 301-309.

Gutzwiller, A., Czegledi, L., Stoll, P., Bruckner, L. 2007. Effects of *Fusarium* toxins on growth, humoral immune response and internal organs in weaner pigs, and the efficacy of apple pomace as an antidote. *J. Anim. Physiol. Anim. Nutr*. 91, 432-438.

Harper, A.F., Estienne, M.J., Meldrum, J.B., Harrell, R.J., Diaz, D.E. 2010. Assessment of a hydrated sodium calcium aluminosilicate agent and antioxidant blend for mitigation of aflatoxin-induced physiological alterations in pigs. *J. Swine Health Prod*. 18, 282-289.

Huwig, A., Freimund, S., Kappeli, O., Dutler, H. 2001. Mycotoxin detoxication of animal feed by different adsorbents. *Toxicol. Lett.* 122, 179-188.

IARC, 1993. International Agency for Research on Cancer. Monographs on the Evaluation of Carcinogenic Risks to Humans, Some Naturally Occurring Substances, Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, vol. 56. Lyon, International Agency for Research on Cancer.

IARC, 2002. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 82. Lyon, International Agency for Research on Cancer.

Jard, G., Liboz, T., Mathieu, F., Guyonvarc'h, A., Lebrihi, A. 2011. Review of mycotoxin reduction in food and feed: from prevention in the field to detoxification by adsorption or transformation. *Food Addit. Contam.* 28, 1590-1609

Jaynes, W.F., Zartman, R.E., Hudnall, W.H. 2007. Aflatoxin B1 adsorption by clays from water and corn meal. *Appl. Clay Sci*. 36, 197-205.

Jiang, S.Z., Yang, Z.B., Yang, W.R., Wang, S. J., Liu, F.X., Johnston, L.A., Chi, F., Wang, Y. 2012. Effect of purified zearalenone with or without modified montmorillonite on nutrient availability, genital organs and serum hormones in post-weaning piglets. *Livest. Sci.* 144, 110-118.

Jouany, J.P. 2007. Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. *Anim. Feed Sci. Technol*. 137, 342-362.

Kabak, B., Dobson, A.D.W., Var, I. 2006. Strategies to prevent mycotoxin contamination of food and animal feed: a review. *Crit. Rev. Food Sci. Nutr*. 46, 593-619.

Karlovsky, P. 2011. Biological detoxification of the mycotoxin deoxynivalenol and its use in genetically engineered crops and feed additives. *Appl. Microbiol. Biotechnol*. 91, 491-504.

Kolosova, A., Stroka, J. 2011. Substances for reduction of the contamination of feed by mycotoxins. A review. *World Mycotoxin J*. 4, 225-256.

Kong, C., Shin, S.Y., Kim, B.G. 2014. Evaluation of mycotoxin sequestering agents for aflatoxin and deoxynivalenol: an *in vitro* approach. *SpringerPlus*, 3, 346-351.

Kosicki, R., Błajet-Kosicka, A., Grajewski, J., Twarużek, M. 2016. Multiannual mycotoxin survey in feed materials and feedingstuffs. *Anim. Feed Sci. Technol*. 215, 165-180.

Kyriakys, S.C., Papaioannou, D.S., Alexopoulos, C., Polizopoulou, Z., Tzika, E.D., Kyriakis, C.S. 2002. Experimental studies on safety and efficacy of the dietary use of a clinoptilolite-rich tuff in sows: a review of recent research in Greece. *Micropor. Mesopor. Mat*. 51, 65-74.

Li, Z., Yang, Z.B., Yang, W.R., Wang, S.J., Jiang, S.Z., Wu, Y.B. 2012. Effects of feedborne *Fusarium* mycotoxins with or without yeast cell wall adsorbent on organ weight, serum biochemistry, and immunological parameters of broiler chickens. *Poult. Sci.* 91, 2487-2495.

Liu, Y.L., Meng, G.Q., Wang, H.R., Zhu, H.L., Hou, Y.Q., Wang, W.J., Ding, B.Y. 2011. Effect of three mycotoxin adsorbents on growth performance, nutrient retention and meat quality in broilers fed on mould-contaminated feed. *British Poult. Sci.* 52, 255-263.

Magnoli, A.P., Monge, M.P., Miazzo, R.D., Cavaglieri, L.R., Magnoli, C.E., Merkis, C.I., Cristofolini, A.L., Dalcero, A.M., Chiacchiera, S.M. 2011. Effect of low levels of aflatoxin B-1 on performance, biochemical parameters, and aflatoxin B1 in broiler liver tissues in the presence of monensin and sodium bentonite. *Poult. Sci*. 90, 48-58.

Marin, S., Ramos, A.J., Cano-Sancho, C., Sanchis, V. 2013. Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food Chem. Toxicol*. 60, 218-237.

Martin, L.M., Wood, K.M., McEwen, P.L., Smith, T.K., Mandell, I.B., Yannikouris, A., Swanson, K.C. 2010. Effects of feeding corn naturally contaminated with *Fusarium* mycotoxins and/or a modified yeast cell wall extract on the performance, immunity and carcass characteristics of grain-fed veal calves. *Anim. Feed Sci. Technol*. 159, 27-34.

Matejova, I., Svobodoba, Z., Vakula, J., Mares, J., Modra, H. 2017. Impact of mycotoxins on aquaculture fish species: A review. *J. World Aquacult. Soc*. 48, 186- 200.

Medina, A., Rodríguez, A., Sultan, Y., Magan, N. 2015. Climate change factors and *Aspergillus flavus*: effects on gene expression, growth and aflatoxin production. *World Mycotoxin J*. 8, 171-179.

Meissonnier, G.M., Oswald, I.P., Galtier, P. 2005. Aflatoxicosis in swine - A bibliographic review of clinical cases and experimental data. *Rev. Méd. Vét.* 156, 591-605.

Mezes, M., Balogh, K., Toth, K. 2010. Preventive and therapeutic methods against the toxic effects of mycotoxins – a review. *Acta Vet. Hung.* 58, 1-17.

Miazzo, R., Peralta, M.F., Magnoli, C., Salvano, M., Ferrero, S., Chiacchiera, S.M., Carvalho E.C., Rosa C.A., Dalcero, A. 2005. Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin. *Poult. Sci*. 84, 1-8.

Miraglia, M., Marvin, H.J., Kleter, G.A., Battilani, P., Brera, C., Coni, E., Cubadda, F., Croci, L., De Santis, B., Dekkers, S., Filippi, L., Hutjes, R.W., Noordam, M.Y., Pisante, M., Piva, G., Prandini, A., Toti, L., van den Born, G.J., Vespermann, A. 2009. Climate change and food safety: An emerging issue with special focus on Europe. *Food Chem. Toxicol. 47*, 1009-1021.

Mohaghegh, A., Chamania, M., Shivazada, M., Sadeghia, A.A., Afzalib, N. 2017. Effect of esterified glucomannan on broilers exposed to natural mycotoxin-contaminated diets. *J Appl. Anim. Res.* 45, 285-291.

Monbaliu, S., Van Poucke, C., Detavernier, C., Dumoulin, F., Van De Velde, M., Schoeters, E., Van Dyck, S., Averkieva, O., Van Peteghem, C., De Saeger, S. 2010. Occurrence of mycotoxins in feed as analyzed by a multi-mycotoxin LC-MS/MS method. *J. Agric. Food Chem.* 58, 66-71.

Morgavi, D.P., Riley, R.T. 2007. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with *Fusarium* toxins. *Anim. Feed Sci. Technol*. 137, 201-212.

Neeff, D.V., Ledoux, D.R., Rottinghaus, G.E., Bermudez, A.J., Dakovic, A., Murarolli, R.A., Oliveira, C.A.F. 2013. *In vitro* and *in vivo* efficacy of a hydrated sodium calcium aluminosilicate to bind and reduce aflatoxin residues in tissues of broiler chicks fed aflatoxin B1. *Poult. Sci*. 92, 131-137.

Nesic, K., Resanovic, R., Nesic, V., Sinovec, Z. 2008. Efficacy of mineral and organic adsorbent in alleviating harmful effects of zearalenone on pigs performance and health. *Acta Vet. Beograd*. 58, 211-219.

Papaioannou, D., Katsoulos, P.D., Panousis, N., Karatzias, H. 2005. The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: A review. *Micropor. Mesopor. Mat.* 84, 161-170.

Pappas, A.C., Tsiplakou, E., Georgiadou, M., Anagnostopoulos, C., Markoglou, A.N., Liapis, K., Zervas, G. 2014. Bentonite binders in the presence of mycotoxins: results of *in vitro* preliminary tests and an *in vivo* broiler trial. *Appl. Clay Sci.* 99, 48-53.

Paulick, M., Rempe, I., Kersten, S., Schatzmayr, D., Schwartz-Zimmermann, H.E., Danicke, S. 2015. Effects of increasing concentrations of sodium sulfite on deoxynivalenol and

deoxynivalenol sulfonate concentrations of maize kernels and maize meal preserved at various moisture content. *Toxins,* 7, 791-811.

Pfohl-Leszkowicz, A., Hadjeba-Medjdoub, K., Ballet, N., Schrickx, J., Fink-Gremmels, J. 2015. Assessment and characterisation of yeast-based products intended to mitigate ochratoxin exposure using *in vitro* and *in vivo* models. *Food Addit. Contam.* 32, 604-616.

Phillips, T.D., Afriyie-Gyawu, E., Williams, J., Huebner, H., Ankrah, N.A., Ofori-Adjei, D., Jolly, P., Johnson, N., Taylor, J., Marroquin-Cardona, A. Xu, L., Tang, L., Wang, J.S. 2008. Reducing human exposure to aflatoxin through the use of clay: A review. *Food Addit. Contam.* 25, 134-145.

Pinotti, L., Ottoboni, M., Giromini, C., Dell'Orto, V., Cheli, F. 2016. Mycotoxin Contamination in the EU Feed Supply Chain: A Focus on Cereal Byproducts. *Toxins,* 8, 45.

Pinton, P., Tsybulskyy, D., Lucioli, J., Laffitte, J., Callu, P., Lyazhri, F., Grosjean, F., Bracarense, A.P., Kolf-Clauw, M., Oswald, I.P. 2012. Toxicity of deoxynivalenol and its acetylated derivatives on the intestine: differential effects on morphology, barrier function, tight junctions proteins and MAPKinases. *Toxicol. Sci.* 130, 180-190.

Ramos A. J., Fink-Gremmels J., Hernández E. 1996a. Prevention of toxic effects of mycotoxins by means of nonnutritive adsorbent compounds. *J. Food Prot*. 59, 631-641.

Ramos, A.J., Hernández, E. 1996. *In vitro* aflatoxin adsorption by means of a montmorillonite silicate. A study of absorption isotherms. *Anim. Feed Sci. Technol*. 62, 263-269.

Ramos, A.J., Hernández, E., 1997. Prevention of aflatoxicosis in farm animals by means of hydrated sodium calcium aluminosilicate addition to feedstuffs: A review. *Anim. Feed Sci. Technol.* 65, 197-206.

Ramos, A.J., Hernández, E., Pla-Delfina, J.M., Merino, M. 1996b. Intestinal absorption of zearalenone and *in vitro* study of non-nutritive sorbent materials. *Int. J. Pharm.* 128, 129-137.

Rawal, S., Kim, J.E., Coulombe, R. Jr. 2010. Aflatoxin B_1 in poultry: Toxicology, metabolism and prevention. *Res. Vet. Sci*. 89, 325-331.

Ringot, D., Lerzy, B., Chaplain, K., Bonhoure, J.P., Auclair, E., Larondelle, Y. 2007. *In vitro* biosorption of ochratoxin A on the yeast industry by-products: comparison of isotherm models. *Bioresour. Technol.* 98, 1812-1821.

Rodrigues, I., Naehrer, K. 2012. Prevalence of mycotoxins in feedstuffs and feed surveyed worldwide in 2009 and 2010. *Phytopatol. Mediterr*. 51, 175-192.

Rodríguez-Carrasco, Y., Ruiz, M.J., Font, G., Berrada, H. 2013. Exposure estimates to *Fusarium* mycotoxins through cereals intake. *Chemosphere*. 93, 2297-2303.

Ruiz, M.J., Macáková, P., García, A.J., Font, G. 2011. Cytotoxic effects of mycotoxin combinations in mammalian kidney cells. *Food Chem. Toxicol*. 49, 2718-2724.

Sabater-Vilar, M., Malekinejad, H., Selman, M.H.J., van der Doelen, M.A.M., Fink-Gremmels, J. 2007. *In vitro* assessment of adsorbents aiming to prevent deoxynivalenol and zearalenone mycotoxicoses. *Mycopathologia*, 163, 81-90.

Santos, R.R., Vermeulen, S., Haritova, A., Fink-Gremmels, J. 2011. Isotherm modeling of organic activated bentonite and humic acid polymer used as mycotoxin adsorbents. *Food Addit. Contam.* 28, 1578-1589.

Shetty, P.H., Jespersen, L. 2006. *Saccharomyces cerevisiae* and lactic acid bacteria as potential mycotoxin decontaminating agents. *Trends Food Sci. Technol.* 17, 48-55.

Stanciu, O., Juan, C., Miere, D., Loghin, F., Mañes, J. 2017. Occurrence and co-occurrence of *Fusarium* mycotoxins in wheat grains and wheat flour from Romania. *Food Control*, 73, 147-155.

Streit, E., Naehrer, K., Rodrigues, I., Schatzmayr, G. 2013. Mycotoxin occurrence in feed and feed raw materials worldwide: Long-term analysis with special focus on Europe and Asia. *J. Sci. Food Agric*. 93, 2892-2899.

Streit, E., Schatzmayr, G., Tassis, P., Tzika, E., Marin, D., Taranu, I., Tabuc, C., Nicolau, A., Aprodu, I., Puel, O., Oswald, I.P. 2012. Current Situation of Mycotoxin Contamination and Co-occurrence in Animal Feed-Focus on Europe. *Toxins* 4, 788-809.

Swamy H.V.L.N, Smith, T.K., MacDonald, E.J., Karrow, N.A., Woodward, B., Boermans, H.J. 2003. Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on growth and immunological measurements of starter pigs, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. *J. Anim. Sci.* 81, 2792-2803.

Swamy, H. V. L. N., Smith, T.K., Cotter, P.F., Boermans, H.J., Sefton, A.E. 2002. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on production and metabolism in broilers. *Poult. Sci.* 81, 966-975.

Thieu, N. Q., Ogle, B., Pettersson, H. 2008. Efficacy of bentonite clay in ameliorating aflatoxicosis in piglets fed aflatoxin contaminated diets. *Trop. Anim. Health Prod*. 40, 649-656.

Vekiru, E., Fruhauf, S., Sahin, M., Ottner, F., Schatzmayr, G., Krska, R. 2007. Investigation of various adsorbents for their ability to bind aflatoxin B1. *Mycotox. Res*. 23, 27-33.

Vidal, A., Marín, S., Ramos, A.J., Cano-Sancho, G., Sanchis, V. 2013. Determination of aflatoxins, deoxynivalenol, ochratoxin A and zearalenone in wheat and oat based bran supplements sold in the Spanish market. *Food Chem. Toxicol.* 53, 133-138.

Vila-Donat P., Marín, S., Sanchis, V., Ramos, A.J. Personal Communication a. Evaluación de nuevos adsorbentes multimicotoxínicos basados en bentonitas para alimentación animal. XXII Congreso Español de Toxicología y VI Iberoamericano (AETOX), June 2017, Valencia (Spain). *Rev. Toxicol*. 34, 21

Vila-Donat, P., Marín, S., Sanchis, V., Ramos, A.J. Personal Communication b. *In vitro* assessment of new efficient mycotoxin adsorbents based on bentonites. 31st EFFOST International Conference, November 2017, Sitges (Spain). Book of abstracts, P2-196.

Voss, K.A., Smith, G.W., Haschek, W.M. 2007. Fumonisins, toxicokinetics, mechanism of action and toxicity. *Anim. Feed Sci. Technol.* 137, 299-325.

Wang, J.P., Chi, F., Kim, I.H. 2012. Effects of montmorillonite clay on growth performance, nutrient digestibility, vulva size, faecal microflora, and oxidative stress in weaning gilts challenged with zearalenone. *Anim. Feed Sci. Technol*. 178, 158-166.

Weaver, A.C, See, M.T., Kim, S.W. 2014. Protective Effect of Two Yeast Based Feed Additives on Pigs Chronically Exposed to Deoxynivalenol and Zearalenone. *Toxins*. 6, 3336-3353.

Weaver, A.C., See, M.T., Hansen, J.A., Kim, Y.B, De Souza, A.L.P., Middleton, T.F., Kim, S.W. 2013. The Use of Feed Additives to Reduce the Effects of Aflatoxin and Deoxynivalenol on Pig Growth, Organ Health and Immune Status during Chronic Exposure. *Toxins*. 5, 1261-1281.

Wielogórska, E., MacDonald, S., Elliot C.T. 2016. A review of the efficacy of mycotoxin detoxifying agents used in feed in light of changing global environment and legislation. *World Mycotoxin J.* 9, 419-433.

Wozny, M., Obremski, K., Jakimiuk, E., Gusiatin, M., Brzuzan, P. 2013. Zearalenone contamination in rainbow trout farms in north-eastern Poland. *Aquaculture*. 416, 209-211.

Yegani, M., Smith, T.K., Leeson, S., Boermans, H.J. 2006. Effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism of broiler breeders. *Poult. Sci*. 85, 1541-1549.

Yunus, A.W., Valenta, H., Abdel-Raheem, S.M., Döll, S., Dänicke, S., Böhm, J. 2010. Blood plasma levels of deoxynivalenol and its de-epoxy metabolite in broilers after a single oral dose of the toxin. *Mycotoxin Res*. 26, 217-220.

Zachariasova, M., Dzuman, Z., Veprikova, Z., Hajkova, K., Jiru, M., Vaclavikova, M., Zachariasova, A., Pospichalova, M., Florian, M., Hajslova, J. 2014. Occurrence of multiple mycotoxins in European feedingstuffs, assessment of dietary intake by farm animals. *Anim. Feed Sci. Technol.* 193, 124-140.

Zain, M.E. 2011. Impact of mycotoxins on humans and animals. *J. Saudi Chem. Soc.* 15, 129-144.

Zinedine, A., Soriano, J.M., Moltó, J.C., Mañes, J. 2007. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone, an oestrogenic mycotoxin. *Food Chem. Toxicol*. 45, 1-18.

Figure and Table legends

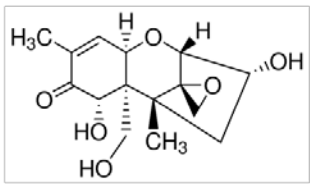
Figure 1. Chemical structures of major mycotoxins in animal feed.

Table 1. Overview of the most relevant mycotoxins in animal farms.

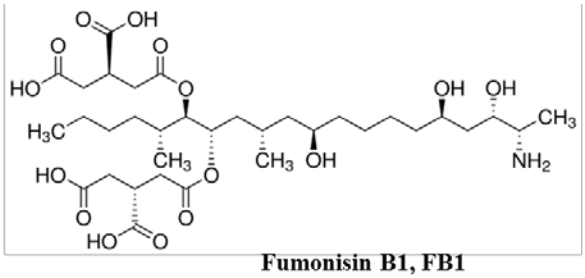
Table 2. Maximum levels for AFB1 in feed and feedingstuffs (Directive 2002/32/CE, amended by Directive 2003/100).

Table 3. Guidance values for DON, ZEN, FBs and OTA in feedstuffs (2006/576/EU) and for T-2 and HT-2 (2013/165/EU).

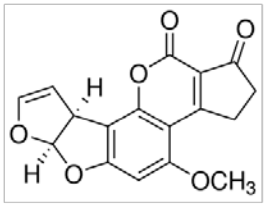
Table 4. Multi-binding efficiencies of various mycotoxin adsorbents in different animal species.



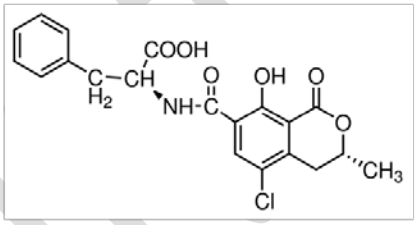
Deoxynivalenol, DON
MW: 296. 3



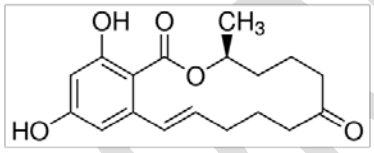
Fumonisin B1, FB1
MW: 721.8



Aflatoxin B1, AFB1
MW: 312.2



Ochratoxin A, OTA
MW: 403.8



Zearalenone, ZEN
MW: 318.3

Figure 1

Table 1.

Major classes of mycotoxins	Most relevant representatives in animal feed	Mycotoxin-producing fungi	Main effects observed in animals	References
Aflatoxins	AFB1, AFB2, AFG1, AFG2	*Aspergillus flavus*, *Aspergillus parasiticus*	Liver disease (hepatotoxic), carcinogenic and teratogenic effects.	Magnoli et al., 2011; Rawal et al., 2010; Streit et al., 2012.
Trichothecenes	DON, 3- or 15-Ac-DON, NIV (type B) T-2, HT-2 (type A)	*Fusarium graminearum*, *Fusarium sporotrichioides*, *Fusarium poae*, *Fusarium equiseti*	Immunologic effects, haematological changes, digestive disorders (diarrhoea, reduced FI) dermatitis, oral lesions, haemorrhages of intestinal tissues, edema.	Awad et al., 2006; Bryden, 2012; Pinton et al., 2012.
Zearalenone	ZEN	*Fusarium graminearum*	Estrogenic effects (edema of vulva, enlargement of uterus), atrophy of ovaries and testicles, abortion.	Wozny et al., 2013; Zinedine et al., 2007.
Ochratoxins	OTA	*Aspergillus ochraceus*, *Penicillium verrucosum*, *Penicillium viridicatum*	Nephrotoxicity, porcine nephropathy, mild liver damage, immune suppression.	Boudergue et al., 2009; Pfohl-Leszkowicz et al., 2015.
Fumonisins	FB1, FB2	*Fusarium verticillioide*, *Fusarium proliferatum*	PPE (porcine pulmonary edema), ELEM (equine leukoencephalomalacia), nephrotoxicity, hepatotoxicity.	Bouhet and Oswald, 2007; Morgavi and Riley, 2007; Voss et al., 2007.

Aflatoxin B_1 (AFB1), Aflatoxin B_2 (AFB2), Aflatoxin G_1 (AFG1), Aflatoxin G_2 (AFG2), Deoxynivalenol (DON), T-2 toxin (T-2), HT-2 toxin (HT-2), Zearalenone (ZEN), Ochratoxin A (OTA), Fumonisin B_1 (FB1), Fumonisin B_2 (FB2), Feed Intake (FI), 15-acetyldeoxynivalenol (15-Ac-DON), 3-acetyldeoxynivalenol (3-Ac-DON), Nivalenol (NIV).

Table 2.

Feedstuffs	Maximum content of AFB1 in mg/kg*
All feed materials	0.02
Complete feedingstuffs for cattle, sheep and goats with the exception of :	0.02
- Complete feedingstuffs for dairly cattle	0.005
- Complete feedingstuffs for calves and lambs	0.01
Complete feedstuffs for pigs and poultry (except young animals)	0.02
Other complete feedingstuffs	0.01
Complementary feedingstuffs for cattle, sheep and goats (except complementary feedstuffs for dairy animals, calves and lambs)	0.02
Complementary feedingstuffs for pigs and poultry (except young animals)	0.02
Other complementary feedingstuffs	0.005

*relative to a feedingstuff with a moisture content of 12 %

Table 3.

Mycotoxins	Feedstuffs	Guidance value in mg/kg*
OTA	Feed materials: cereals and cereal products	0.25
	Complete and complementary feedstuffs	
	- For pigs	0.05
	- For poultry	0.1
DON	Feed materials:	
	- cereals and cereal products, with the exception of maize by-products	8
	- maize by-products	12
	Complementary and complete feedstuffs with the exception of:	5
	- Complementary and complete feedstuffs for pigs	0.9
	- Complementary and complete feedstuffs for calves (< 4 months), lambs and kids	2
FB1, FB2	Feed materials: maize and maize by-products	60
	Complementary and complete feedstuffs for:	
	- pigs, horses (Equidae), rabbits and pet animals	5
	- fish	10
	- poultry, calves (< 4 months), lambs and kids	20
	- adults ruminants (> 4 months) and mink	50
ZEN	Feed materials:	
	- cereals and cereal products, with the exception of maize by-products	2
	- maize by-products	3
	Complementary and complete feedstuffs:	
	▪ for piglets and gilts (young sows)	0.1
	▪ for sows and fattening pigs	0.25
	▪ for dairy cattle, sheep (including lambs) and goats (including kids)	0.5
T-2, HT-2	Cereal products for feed and compound feed:	
	- oat milling products	0.25
	- other cereal products	0.5
	- compound feed (with the exception of feed for cats)	2

Deoxynivalenol (DON), T-2 toxin (T-2), HT-2 toxin (HT-2), Zearalenone (ZEN), Ochratoxin A (OTA), Fumonisin B_1 (FB1), Fumonisin B_2 (FB2).

*relative to a feedingstuff with a moisture content of 12 %

Table 4.

Species	Feed	Mycotoxins and concentrations (mg/kg)	Adsorbents (% added to contaminated feed)	Duration of the study	Effect of the addition of the AA to the co- contaminated diets	References
Broilers	Corn-soybean meal	AFB1 (0.01 mg/kg) + OTA (0.1 mg/kg) + ZEN (1 mg/kg)	Bentonite (1%)	42 days	Maintained optimum broiler performance.	Pappas et al., 2014
	Contaminated corn	AFB1 (2.5 mg/kg) + FB1 (200 mg/kg)	Sodium bentonite (0.3%)	50 days	Protective effects on gross hepatic changes induced by AFB1.	Miazzo et al., 2005
	Naturally contaminated maize	AFB1 (0.45 mg/kg) + OTA (0.06 mg/kg) + T-2 (0.23 mg/kg)	A: EGM (0.05%), B: HSCAS (0.2%), A+B: EGM+HSCAS (0.1%)	42 days	A: decreased yellowness in breast muscle but no effect on BW and FI B: partially recovered nutrient retention but no effect on BW and FI. A+B: recovered FI, BW and nutrient retention.	Liu et al., 2011
	Naturally contaminated maize	AFB1 (0.168 mg/kg) + OTA (0.0084 mg/kg) + ZEN (0.053 mg/kg) + T-2 (0.032 mg/kg)	EGM (0.05%)	35 days	Increased performance and serum levels of urea nitrogen and decreased the activity of γ-glutamyl transferase. Counteracted the toxic effects produced by mycotoxins present in diet, by alleviating growth depression and reducing organ weights.	Aravind et al., 2003
	Naturally contaminated grains	DON (9.7 mg/kg) + ZEN (0.8 mg/kg) + FA (21.6 mg/kg)	EGM (0.2%)	56 days	No effect on feed consumption, WG, and feed efficiency but counteracted most of the blood parameter alteration and reduced breast muscle redness.	Swamy et al., 2002
	Naturally contaminated corn	AFB1 (0.4273 mg/kg) + DON (0.20631 mg/kg) + OTA (0.0022 mg/kg)	EGM "Mycosorb®" (0.05, 0.1%)	49 days	At 0.05 %, improved the decreased BW gain and FI. At 0.1%, additionally ameliorated the negative impact of mycotoxins on feed conversion ratio.	Mohaghegh et al., 2017
	Naturally contaminated	AFB1 (102.08 mg/kg) + ZEN (281.9 mg/kg) +	YCW (2%)	42 days	Significantly improved the relative weight of the liver, spleen, bursa of	Li et al., 2012

	corn	FB1 (5,874.3 mg/kg) + DON (2,038.9 mg/kg)			Fabricius, and thymus; increased anti-Newcastle disease titers; positive protection effect on the splenic mRNA expression of IL-1β, IL-6, and IFN-γ tested.	
	Naturally contaminated corn	DON (12.6 mg/kg) + ZEN + 15- Ac- DON (lesser amounts)	GM polymer (0.2%)	12 weeks	Prevented the decrease of eggshell, and antibody titers against infectious bronchitis virus.	Yegani et al., 2006
Pigs	Naturally contaminated grains (mostly corn)	DON (5.5 mg/kg) + FA (26.8 mg/kg) + ZEN (0.4 mg/kg) + 15-Ac-DON (0.3 mg/kg)	GM polymer (0.2%)	21 days	Prevented some toxin-induced metabolic changes.	Swamy et al., 2003
	Contaminated wheat	DON (2.1-3.2 mg/kg) + ZEN (0.06-0.25 mg/kg)	Apple pomace (8%)	5 weeks	Alleviated the negative effects of DON on growth.	Gutzwiller et al., 2007
	Naturally contaminated maize	DON (8.6 mg/kg) + ZEN (1.2 mg/kg)	Modified aluminosilicate (0.4%)	36 days	No protective effects were observed	Doll et al., 2005
	Contaminated diets for sows	DON (0.14-0.31 mg/kg) + ZEN (0.16-1.55 mg/kg)	Clinoptilolite-Zeolite (2%)	Duration of a complete sow's reproductive cycle	Beneficial effect on sow's reproductive performance and protection against the detrimental consequences of zearalenone toxicosis.	Kryiakys et al., 2002
	Naturally contaminated corn	DON (4.8 mg/kg) + ZEN (0.3 mg/kg)	A: YCW (2%); B: Yeast fermentation additive (2%)	42 days	A: reduced oxidative stress and internal damage; B: improved the growth performance of pigs by increasing FI and WG	Weaver et al., 2014
	Naturally contaminated corn and barley	AFB1 (0.15 mg/kg) + DON (1.1 mg/kg)	A: Montmorillonite (2%); B: sodium bentonite, sepiolite, yeast (1.5%); C: diatomaceous earth, yeast culture (1.1%)	42 days	A, B: reduced effects caused by mycotoxins on the immune system and the liver, and improved growth. C: reduced liver damage	Weaver et al., 2013
Cattle	Naturally contaminated corn	DON (10.27 mg/kg) + 15-Ac-DON (1.27 mg/kg) + ZEN (1.84 mg/kg)	Modified YCW (1%)	84 days	Mycotoxin levels assayed did not produce negative effects on calves, so effects of the binder could not be proven	Martin et al., 2010

Aflatoxin B_1 (AFB1), Deoxynivalenol (DON), T-2 toxin (T-2), Fusaric acid (FA), Zearalenone (ZEN), Ochratoxin A (OTA), Fumonisin B_1 (FB1), 15-acetyldeoxynivalenol (15-Ac-DON), 3-acetyldeoxynivalenol (3-Ac-DON), Nivalenol (NIV), Esterified Glucomannan (EGM), Hydrated Sodium Calcium Aluminosilicate (HSCAS), Glucomannan polymer (GM), Yeast Cell Wall (YCW), Body Weight (BW), Feed Intake (FI), Weight Gain (WG).