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# A Focus on Aflatoxins in Feedstuffs: Levels of Contamination, Prevalence, Control Strategies, and Impacts on Animal Health

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Andrea Molina Alvarado,  
Rebeca Zamora-Sanabria and  
Fabio Granados-Chinchilla

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## Abstract

Aflatoxins are mold-synthesized secondary metabolites that are ubiquitously present in agricultural commodities, such as cereals which in turn are substantial part of feed formulation. These toxins are capable of causing disease, exert severe toxic effects, and even death in humans and other animals. Aflatoxins are the only mycotoxins with the regulatory framework, hence we present the legal threshold uphold till now by international and regional control organizations. Additionally, herein we discuss worldwide prevalence of aflatoxins in feeds to demonstrate a global issue and major risks involved in toxin contamination. Furthermore, we present recent data regarding negative effects usually presented by food-producing and companionship animals when ingested. Also, we discuss briefly practical approaches to mitigate aflatoxin burden during feed processing focusing in Good Manufacturing Practice (GMP) and hazard analysis critical control point (HACCP) and we include novel approaches reported in literature to decontaminate feed-containing aflatoxins. Finally, we cite the literature so far published describing the effects of changing climate on aflatoxin production and contamination.

**Keywords:** aflatoxins, risk factors, prevalence, animal health effects, mycotoxin sorbents, toxicity, climate change

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## 1. Introduction

Livestock, aquaculture animals, and pets are exposed through dietary contact (i.e., through feedstuff) to toxic fungal metabolites such as mycotoxins. Mycotoxins are low-molecular-weight

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natural products (i.e., small molecules) produced as secondary metabolites by filamentous fungi. Diseases produced by these means are collectively called mycotoxicoses. As with other toxicological syndromes, exposure to mycotoxins may be acute or chronic, veterinary health problems associated with mycotoxin exposure are usually the result of prolonged contact. This review chapter focuses specifically on aflatoxins. Aflatoxins are a group of biochemical substances produced especially by *Aspergillus* species [1]. They are usually found in cereals and grains such as rice, corn, sorghum, millet, and groundnuts during the harvesting, storage, and poor processing conditions [1].

Aflatoxin contamination associated with food or feed is a global problem especially in the tropical and subtropical regions of the world, where warm temperatures and humidity favor the growth of the fungi [2]. Considering its economic and health relevance, we will discuss certain aspects of the relationship of the contaminant with feeds and feed ingredients. Emphasis will be on the fact that animal feed, and ingredients thereof, are situated at the start the food chain and contaminated feed will, therefore, have an adverse impact on the rest of the alimentary web. Animal feedingstuffs quality directly affect animal productivity, health and can have drastic effects on food that is later consumed by humans as final products [3, 4]. Within the context of aflatoxins, we will discuss food chain safety, prevalence in animal feed and regulations. We will also mention risk factors and health effects of aflatoxins on animals, and control and management approaches to reduce them.

## 2. Aflatoxins in animal feed

Aflatoxins can be found worldwide in a variety of food and feed commodities especially cereals; the contamination with aflatoxin-producing fungi and the production of the toxin in the products can occur in the field, during storage, transportation at almost all stages of the production chain. In finished animal feed, the contamination of an ingredient could cause the contamination of an entire feed batch [5]. Furthermore, the introduction of a feedstuff contaminated with aflatoxin-producing fungi could lead to the spoilage of other feed shipments and serves as a fungi source in the feed industry environment difficult to eliminate. This deterioration effect has a significant repercussion in association with the global trade and the international exchange of animal feed and feed ingredients [6]. Co-occurrence of different mycotoxins in finished feed could have profound negative effects on animal and human health, due to the synergistic or additive effect among toxins [6]. The global production of animal feed reached 964 million tons in 2014 [7]. Cereal grains, primarily corn, are widely used as energy source in animal feed for different species. These raw materials represent 50–80% of the animal diet in America and Europe. USA and Brazil are the major corn exporter countries, and Japan and Mexico are the largest importer countries [8]. For example, most of the ingredients used in Malaysia for the production of animal feeds such as cereal grains, soybean meal, corn gluten meal, and soybean meal are imported from Thailand, China, India, Argentina, USA, Australia, and Canada. Mycotoxin contamination of feed caused by poor storage conditions during production and transportation are frequent [9]. In Costa Rica, the animal feed produced is based on corn products and only during 2015 over 764 254 tons of corn products were imported [10].

These are only examples of the importance of global trade for the animal feed industry; in this scenario, the origin of the ingredients and the place and length of storage must be taken into account to make a conclusion about mycotoxin contamination in a region. Furthermore, frequently agricultural commodities (peanuts, corn, and rice) used as feed ingredients originating from tropical and subtropical regions contain high amounts of aflatoxins [6, 11].

### 2.1. Major risk factors for aflatoxins in feedstuffs

As already mentioned above, the contamination of animal feedstuff could take place at different stages throughout the entire food chain. Mycotoxins in feedstuff and finished feed should be monitored from farm-to-fork to assure a safety product for animal and humans. The contamination of cereal grains and other agricultural commodities used in animal feed could occur in the field during the pre-harvest phase during harvest, or in processing stages (postharvest).

In the pre-harvest period, the presence of aflatoxin-producing fungi (and then the production of the toxin) could be influenced and potentiated by different factors such as the plant genetics, e.g. the use of corn germplasm not adapted to local conditions [12]. After that, during the growing and harvesting stages, toxin evolution is predisposed by agricultural practices, including the use of fungicides and pesticides, the use of open-pollinated varieties [13], the contact with aflatoxin-producing fungi or its spores, weather conditions and climate during planting and growing and, finally, insect damage.

Moisture and temperature play a significant role in fungi growth and the production of aflatoxins. Mycotoxin-producing fungi frequently need higher moisture levels (20.0–25.0 g/100 g) for infection during the pre-harvest phase in the field than fungi that proliferate during storage (13.0–18.0 g/100 g) [14]. Agricultural practices that have bearing over crop susceptibility toward infection and contamination include the variety of crops that are planted, the planting date, crop rotation (e.g., avoiding corn as a pre-crop for wheat), and tillage (plowing reduces inoculum from plant residues) [15].

It is worth clarifying that the presence of aflatoxin-producing fungi such as *Aspergillus parasiticus* or *Aspergillus flavus* in plants or the field environment does not necessarily imply the contamination of the crops with the toxin. For the production of aflatoxins, the molds need some stress factors such as nutritional imbalance, drought, or water surplus [16].

Climate plays a relevant role in fungal development and aflatoxin production in crops in the field and during storage [16]. However, in an epidemiological study conducted in our laboratory, 968 samples of animal feed and feed ingredients produced or stored (imported products) in Costa Rica were analyzed for aflatoxins (AFs), in the period 2010–2016. We did not find a direct correlation between aflatoxin concentration and the mean temperature, relative humidity, average rain precipitation, and the number of rainy days for a specific month during the same period in this country [17]. These findings together with the descriptions made by others authors [18] show how difficult it is to predict aflatoxin contamination starting from weather conditions only. The substrate or the ingredient that comprises an animal feed is the most important factor in the fungi growth and mycotoxin production mainly due to its nutritional composition [19].

The fungi growth in cereals and animal feeds after harvest during transportation or storage are also influenced by the temperature, humidity, water activity ( $a_w$ ), the integrity of the grain, insect damage, and the quantity and type of the mycobiota [16]. The increase of the humidity in cereals and feeds during transportation and storage could favor an increment of aflatoxin concentration in these products [2]. Furthermore, the geographic origin, the transportation route, and the area where the feedstuff is stored, and the length of storage together with particular climate conditions will have a significant impact on aflatoxins concentration and animal exposure to this toxin. Due to this, conditions such as geographic region, temperature, humidity, and duration should be taken into account when comparing mycotoxins analysis from raw feed ingredients or in the prediction of aflatoxins contamination in finished feed [19].

Not only cereals *per se* are necessary components of the animal diets but also the by-products of these grains are commonly used to feed animals [20, 21]. Mycotoxins are resistant to majority of food processing techniques. Nevertheless, food processing such as milling, production of ethanol fuels, and beer brewing could affect mycotoxins distribution and concentration [22–24]. These mycotoxin concentrated fractions are usually employed in animal diets as is the case in rice milling process where several by-products (e.g. rice hulls, rice bran, chipped rice, rice polishings) are used as animal feed ingredient [21]. Also, we demonstrated that during the production of cheese, the aflatoxins  $M_1$  is concentrated in whey which is frequently used to feed young animals or as a feed ingredient by its own right [25].

## 2.2. Aflatoxins regulations and surveillance in feedstuffs

Worldwide many countries have regulations concerning the maximum concentration of mycotoxins that could be present in food and feed. However, there are no regulations or guidance levels for all mycotoxins known so far. Aflatoxins, some type A and B trichothecenes, zearalenone, fumonisins, and ochratoxin, compounded the mycotoxins with regulatory or guidance levels, due to their demonstrated toxic effects on animals and humans.

Many aflatoxin regulatory levels are set depending on the particular agricultural commodity or compound feed/food, the type, and age of animal which will consume it and the intended use. Many countries base their regulations on the guidelines established by the European Union (EU) (**Table 1**) or by the United States Food and Drug Administration (FDA) (**Table 1**). Guidelines sometimes differ from each other; in most of the cases, the maximum allowed

### US FDA

<i>Intended use</i>	<i>Grain, grain by-product, feed or other products</i>	<i>AFB<sub>1</sub> maximum level (<math>\mu\text{g kg}^{-1}</math>)</i>
Immature animals	Corn, peanut products, and other animal feeds and ingredients, excluding cottonseed meal	20
Dairy animals, animals not listed above, or unknown use	Corn, peanut products, cottonseed, and other animal feeds and ingredients	20
Breeding cattle, breeding swine and mature poultry	Corn and peanut products	100

<b>US FDA</b>		
<i>Intended use</i>	<i>Grain, grain by-product, feed or other products</i>	<i>AFB<sub>1</sub> maximum level (µg kg<sup>-1</sup>)</i>
Finishing swine 100 pounds or greater in weight	Corn and peanut products	200
Finishing (i.e., feedlot) beef cattle	Corn and peanut products	300
Beef, cattle, swine or poultry, regardless of age or breeding status	Cottonseed meal	300
<b>European Commonwealth</b>		
<i>Matrix</i>	<i>AFB<sub>1</sub> maximum level (µg kg<sup>-1</sup>)</i>	
All feed materials	20	
Complete feedingstuffs for cattle, sheep and goats (except dairy animals)	20	
Complete feedingstuffs for dairy animals	5	
Complete feedingstuffs for calves and lambs	10	
Complete feedingstuffs for pigs and poultry (except young animals)	20	
Other complete feedingstuffs	10	
Complementary feedingstuffs for cattle, sheep, and goats (except complementary feedingstuffs dairy animals, calves, and lambs)	20	
Complementary feedingstuffs for pigs and poultry (except young animals)	20	
Other complementary feedingstuffs	5	

**Table 1.** FDA and EU aflatoxin regulatory guidance for feed and feed ingredients.

content of aflatoxins is lower in the regulations given by the EU than in those granted by the FDA. For example, the limit for aflatoxin in dairy feed is set by the EU in 5 µg kg<sup>-1</sup> and by the FDA in 20 µg kg<sup>-1</sup>.

Finally, other international standards have been implemented by several organizations such as Codex Alimentarius Commission (CAC). There is no CAC standard dealing with aflatoxins in animal feeds but three main policies are included in this matrix including Codex General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995) concerned with hazards in feeds, CAC Codes of Practice for Reduction of Aflatoxins for Milk-producing Animals (CAC/RCP 45-1997), and CAC Codes of Practice for Good Animal Feeding (CAC/RCP 54-2004).

On the other hand, regional legal limits for aflatoxins have also been established; for example, the Southern Common Market (MERCOSUR) and Australia/New Zealand have harmonized maximum limits. Other regional bodies such as the Association of Southeast Asian Nations (ASEAN), the Economic Community of West African States (ECOWAS), and the Common Market for Eastern and Southern Africa (COMESA) are in the process of harmonizing legal thresholds.

### 2.3. Aflatoxins prevalence in animal feed and animal feed ingredients

In the analyses of the aflatoxin prevalence in finished feed, the difference in the raw material available in the diverse world regions, the difference in the nutritional requirements (energy, proteins, mineral, and vitamins) of each animal species, and the global trade of feedstuff should be taken into account. Ingredient diversity in a feed formulation is crucial for the livestock industry. Feed costs account for two-thirds or more of total live costs in pig and poultry production [19, 26, 27].

Country	Commodity	Number of samples	Total AF incidence, % (AFB <sub>1</sub> )	Total AF mean concentration, $\mu\text{g kg}^{-1}$ (AFB <sub>1</sub> )	Maximum, $\mu\text{g kg}^{-1}$ (AFB <sub>1</sub> )	Reference
Global survey (America/Europa/Asia)	Corn, soybean, wheat and finished feed	4627	33	21	6105	[28]
Global survey (Myanmar)	Various feed	11,967	26	57	6323	[148]
Global survey (Vietnam)	Corn	10,172	27	16	6105	[149]
<b>Africa</b>						
Africa (South Africa, Nigeria, Kenya and Ghana)	Grains, feed	177	47	42	556.4	[150]
Ethiopia	Dairy feed	156	(100)	–	(419)	[151]
Jordan	Poultry feed ingredients	105	(19.04)	–	(17.06)	[40]
Jordan	Poultry feed	52	(24)	–	(12.7)	[40]
D.R. Congo	Corn	50	32 (32)	10.33–20.64	103.89 (51.23)	[152]
Kenya	Dairy feed and forages	74	(56)	47.84	147.86	[153]
Rwanda	Animal feed	27	–	100.4–168.6	265	[154]
South-Western Nigeria	Fish feed	94	(92)	–	(826.98)	[155]
South Africa	Compound feeds	92	30	9.0	(71.8)	[156]
Malawi	Corn	90	20.1	8.3	140	[157]
<b>America</b>						
North America	Finished feed	21	24	7	56	[28]
South America	Finished feed	203	26	2	83	[28]
Argentina	Poultry feed	49	86	2.68	37.67	[158]
Argentina	Fish feed	28	50	2.82	8.91	[159]
Brazil	Corn	148	4–23	3.1–16.37	49.9	[160]

Country	Commodity	Number of samples	Total AF incidence, % (AFB <sub>1</sub> )	Total AF mean concentration, µg kg <sup>-1</sup> (AFB <sub>1</sub> )	Maximum, µg kg <sup>-1</sup> (AFB <sub>1</sub> )	Reference
Brazil	Corn	74	(16)	(<0.8)	(3)	[161]
Brazil	Poultry feed	36	(0)	(<0.8)	(<0.8)	[161]
Brazil	Fish feed, soybean bran, corn bran, other cereals	54	16.7–60	1.1–7.4	19.1	[162]
Costa Rica	Feed and feed ingredients	968	23.9	–	290.4	[36]
Costa Rica	Dairy feed	112	21	20.6	439.2	[17]
Venezuela	Pig feed	23	65 (26)	–	6.84	[163]
<b>Asia</b>						
North Asia	Finished feed	622	20	5	225	[150]
South-East Asia	Finished feed	465	81	23	431	[150]
South Asia	Finished feed	127	95	91	2454	[150]
China	Feed and feed ingredients	127	(63–100)	3.4–20	18.1	[164]
India	Livestock feed	48	(33.3)	32	60	[165]
India	Feed ingredients	49	(24.5)	62	–	[165]
Korea	Poultry feed	20	100 (100)	0.56 (0.38)	1.86 (1.70)	[81]
Pakistan	Poultry feed ingredients	77	(60)	(37.62)	(56)	[166]
Pakistan	Poultry feed	410	(44.39)	(23.75)	(78)	[166]
Pakistan	Poultry feed					
<b>Europe</b>						
Central Europe	Finished feed	45	2	0	1	[28]
Southern Europe	Finished feed	47	66	3	103	[28]
Turkey	Feedstuff	76	(26.32)	(1.02)	(11.37)	[33]
Turkey	Feed	30	(56.66)	(0.26)	(3.31)	[33]
Turkey	Dairy cow feed	76	26.3 (26.3)	2.74 (2.25)	8.43 (6.90)	[29]
Turkey	Cattle and lamb-calf feed	180	60	10.72	116.83	[30]
<b>Oceania</b>						
Oceania	Finished feed	75	9	0	9	[28]
Oceania	Wheat	109	5	2.0	30	[28]
Oceania	Corn	11	18	3.0	5	[28]

**Table 2.** Aflatoxin occurrence in feed and feed ingredients worldwide (data published 2012–2017).



There are highly sensitive methods for the analysis of aflatoxins; this could lead to the observation of a high percentage of aflatoxin positive samples in surveys that are not always directly related with a high risk for animals and human health. However, the synergistic/additive effect of some mycotoxins should be taken into account even in the case of low aflatoxin concentrations. **Table 2** shows a summary of aflatoxin surveys data worldwide in feed and feed ingredients published between January 2012 and February 2017.

Between January 2009 and December 2011, Rodrigues and Naehrer carried out a survey on mycotoxins occurrence worldwide in which 4 627 samples of corn, soybean meal, wheat, and finished feed were analyzed [28]. The global prevalence of aflatoxin positive samples and the mean concentration in this survey were 33% and 21  $\mu\text{g}/\text{kg}$ , respectively; some of the results of this study are shown in **Table 2**. In this review, the major percentage of positive samples in finished feed found in South Asia and South-East Asia were 95 and 81% with a mean concentration of 91 and 23  $\mu\text{g kg}^{-1}$ , respectively. Furthermore, in finished feed in South Asia, an extremely high level of aflatoxin (2 454  $\mu\text{g kg}^{-1}$ ) was found. In addition, in some regions of Asia the presence of aflatoxins in corn has been found to be as high as 82% of positive samples. Soybean meal showed a relatively minor susceptibility to aflatoxin contamination.

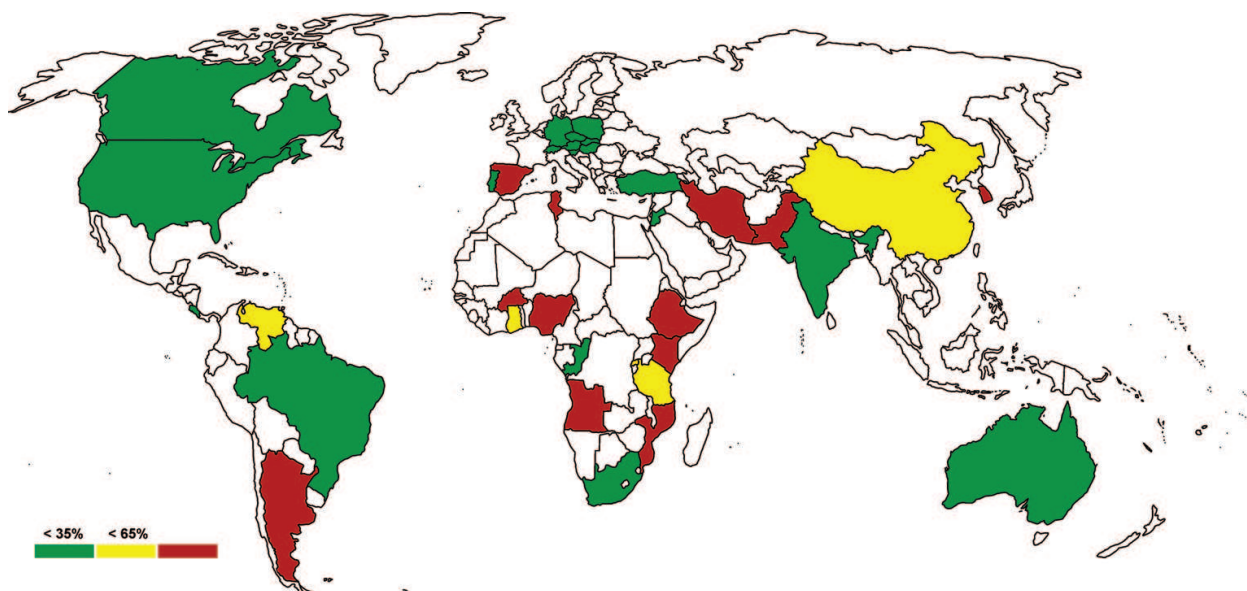
Another example of a global mycotoxins survey was carried out by Kovalsky et al., between 2012 and 2015, in which 1 113 samples of finished feed, corn, and corn silage were analyzed [6]. The authors found that the majority of samples showed an aflatoxin concentration below established guidelines for animal feed, and only a few samples from Africa and Europe presented levels exceeding the 20  $\mu\text{g kg}^{-1}$  limit.

There also a few recent national surveys in regards to mycotoxins occurrence in animal feed; some of their most relevant results are summarized here and in **Table 2**. A recent study in Turkey by Sahin et al. found that from  $n = 76$  cattle feed samples, 26.3% of them exhibited some level, 26.3% of samples exhibited some level of contamination [29], with only two samples exceeding 5  $\mu\text{g kg}^{-1}$ . They did not detect any aflatoxins in ingredients such as sugar beet pulp, alfalfa silage, vetch silage, wheat bran, straw, and cottonseed samples. Kocasari et al. analyzed several toxins including aflatoxins in dairy cattle, beef cattle, and lamb-calf feed ( $n = 180$  each) and found that 61.7% ( $n = 37$ ), 55% ( $n = 33$ ), and 63.3% ( $n = 38$ ), respectively, contained considerable levels of aflatoxins ranging from 3.82 to 116.83  $\mu\text{g kg}^{-1}$  [30]. However, it is important to indicate that the data were gathered using a screening assay. There is evidence, including our own, that seems to indicate that when ELISA is substituted by a confirmatory method such as HPLC, prevalence both in percentage and maximum values attained usually decrease probably due to issues with sensitivity and removal of possible false positive results. For example, Ghali et al. detected aflatoxins in 76.4% ( $n = 58$ ) of the sorghum samples analyzed with an average level of 22.3–20.4  $\mu\text{g kg}^{-1}$  using ELISA [31]. Meanwhile, the same research group found 62% prevalence in sorghum ( $n = 58/93$ ) using HPLC [32].

In another study, AFB<sub>1</sub> was detected in 34.9% ( $n = 37/106$ ) feedstuff and feed samples up to levels of 11.4  $\mu\text{g kg}^{-1}$  [33]. A study conducted by Warth et al. in Burkina Fasso and Mozambique

found a prevalence of feed samples assayed of 100% ( $n = 4/4$ ) and 60 ( $n = 6/10$ ), respectively [34]. The same research group also analyzed corn and sorghum samples from this region with incidences as high as 50% ( $n = 13/26$ ). It is relevant to note that, in this type of assays, a small sample number may hinder reaching a conclusion regarding the region tested. However, it should be taken into account that minor subsets are usual during these types of surveys considering the costs of such analysis, especially those based on HPLC assays. Other research groups have also reported prevalence data from different countries in dairy feed including: Portugal (22% [35]), Costa Rica (33% [36]), China (42% [37]), Tanzania (65% [38]), and Iran (82.5% [39]) (see **Figure 1**). These differences might be due to geographical differentiation, climate, and seasonal variations, feeding systems applied, farm management, and feed storage practices. Research indicates that stricter vigilance systems encourage feed industry to have control over the ingredients used and better administration and prevalence to diminish [17, 40].

Elevated levels of contamination can be achieved if wrong management of feed ingredients has happened at any point during harvesting, storing, or processing. For example, when Kana et al. analyzed corn and feeds in central Africa, in this study, corn was found to be a relevant source of aflatoxins and the mean values of moisture (14.1 g/100 g) for this ingredient was significantly higher when compared to other commodities tested [2]. In the case of Costa Rica, for example,  $n = 15$  samples, recollected along the country during the first trimester of 2016, were found to average  $(13.29 \pm 0.28)$  g/100 g of the nutrient. In this regard, current climate change is expected to affect the behavior of aflatoxigenic fungi and contamination of crops, an excellent review regarding how climate changes mycotoxin behavior was written by Paterson and Lima [41].



**Figure 1.** Worldwide prevalence for aflatoxin, expressed as percentages. Based on scientific reports from each country.

### 3. Effects of aflatoxins on food-producing animals

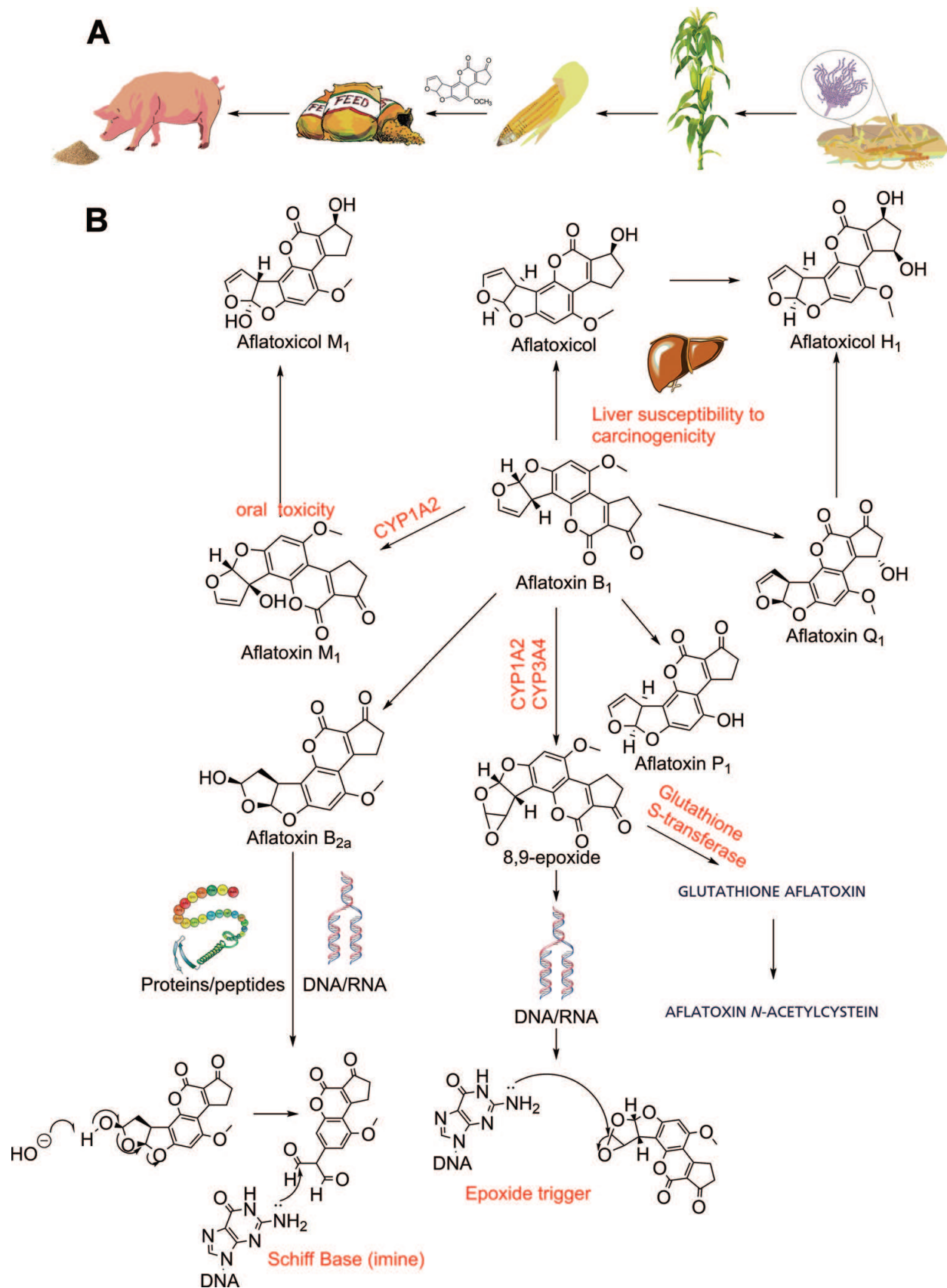
Dietary aflatoxins have shown detrimental effects on animal health and production. The most common exposure route occurs by ingestion of contaminated food. For example, fungal growth under right conditions may carry the genetic battery for toxin production and can contaminate cereals (e.g., corn kernels) which are used as a feed ingredient and, in turn, reach animal farms (**Figure 2A**). Other exposition routes include dermal contact and inhalation. Aflatoxins can affect animals either individually or additively (in the presence of more than one mycotoxin) and may affect various organs and systems [42].

Mycotoxins have a substantial economic impact because all participants of the production chain as farmers, cereals and grains producers, handlers and distributors, crop processors, and consumers suffer losses. Direct effects include increased veterinary care costs, reduced livestock production, and the continuous detriment of food and feed safety features. Also, public health should be another consideration because of the presence of dangerous and undesirable contaminants in animal products.

The disease called aflatoxicosis causes acute and chronic presentation in animals. Acute aflatoxicosis causes death and chronic aflatoxicosis results in cancer, toxicity, and immune suppression. The liver is the primary target organ. AFB<sub>1</sub> is a potent carcinogen [43] by bio-activation of cytochrome P<sub>450</sub> in the liver and AFB<sub>1</sub>-8,9-epoxide (AFBO) production. AFBO is needed for carcinogenic and toxic activity [44].

Aflatoxins susceptibility depends on species, age, gender, and nutrition; there are individual variations in the rate of activation of aflatoxins in various species. Metabolism of AFB<sub>1</sub> involves oxidative reactions by members of the CYP450 family of isoenzymes. There is a variety of metabolizing enzymes in animal species. In poultry species, CYP2A6, CYP3A37, CYP1A5, and CYP1A1 play a significant role in the biotransformation of AFB<sub>1</sub> [45, 46]. In humans, CYP3A4 in the liver and CYP2A13 in the lung have significant activity in metabolizing AFB<sub>1</sub> to AFBO (**Figure 2B**). The rate of AFBO formation and its conjugation with glutathione to reduce the toxicity by glutathione-S-transferase (**Figure 2B**), seem to be an important parameter in interspecies and individual differences [47, 48]. Hence, AFB<sub>1</sub> can cause hepatocellular carcinomas (**Figure 2B**). Cytochrome P<sub>450</sub> involvement, 1A2 (responsible for AFM<sub>1</sub> biosynthesis) and 3A4 result in epoxide formation that leads to non-enzymatic oxidations which turn DNA into a mutagenic prone DNA adduct (encompassing mutations of p53 [activation of ras protooncogenes], leading to mutagenicity) (**Figure 2B**). Ultimately, the DNA adduct is unstable and suffers renal elimination, for example, through conversion to aflatoxin *N*-acetylcysteine.

Rabbits are among the most sensitive animals to the toxic effects of this contaminant, followed by ducks, turkeys, and chickens which are still very sensitive, fish and swine are somewhat susceptible, and cattle and sheep are the most resistant. There are differences between genders, Lozano and Díaz reported male birds to produce more AFBO than females; turkey and duck yield more than chickens and quails [49]. Younger animals are more sensitive to AFB<sub>1</sub> than older individuals [46].



**Figure 2.** (A) Representation of the usual aflatoxin contamination route for grains and (B) several steps of aflatoxin metabolism.

Diet may have both positive and adverse effects on aflatoxin toxicity. Unfavorable results vary and depend on the frequency and source of the contaminated feed ingredients used, the inclusion percentage in the feed, the exposition period, animal species, gender, and age. Some diet components can act positively by exclusion, sorbent mechanisms, and reduction of AFB<sub>1</sub> bioavailability in the gastrointestinal tract [50]. Burkina et al. reported some phytochemicals in nutrition may act inhibiting the enzymes catalyzing AFBO synthesis [51].

The diagnosis of aflatoxins as etiological agents is trying even when mycotoxins are detected. Isolation and confirmation of mycotoxigenic fungal species in food and feeds do not, necessarily, indicate the presence of mycotoxins. Techniques for qualitative and quantitative analysis of mycotoxins vary in sensitivity and accuracy. Sampling could be complicated because there are myriad of factors affecting the production, distribution, or presence of mycotoxins; several products can be contaminated and sometimes it is not easy to identify which one is involved specifically. Also lesions and symptoms in acute and chronic aflatoxicosis are unspecific (immunosuppression, decreased weight gain, hepatic and kidney lesions, and death) and could be caused by other types of agents.

Appropriate diagnostic criteria, reliable sampling, and laboratory testing are still needed to select a correct approach. Prevention of mycotoxins contamination in animal feed is required to avoid losses in animal production and effects in public health.

### 3.1. Effects on pigs

Aflatoxins cause detrimental effects in health and production in swine. Reduction in weight gain and feed intake are among the first symptoms reported. Many researchers have also described diarrhea, bloody feces, and an increase in liver, kidney, spleen, and pancreas size [52–55].

Immune response to aflatoxins has been variable; intake between 120 and 180  $\mu\text{g}$  of AFB<sub>1</sub>  $\text{kg}^{-1}$  of feed in combination with deoxynivalenol may not result in altered immune health [54, 56]. However, altered serum globulin patterns were reported by Mok et al. [55]. Low level of AFB<sub>1</sub> dysregulates the antigen-presenting capacity of porcine dendritic cells; it could explain the immunotoxicity of this mycotoxin [57].

Increased activities of liver-specific enzymes, abnormal histology, increased serum alkaline phosphatase, and  $\gamma$ -glutamyltransferase has been observed in exposed pigs [54, 55].

Pregnant sows treated with 1–3  $\text{mg kg}^{-1}$  of AFB<sub>1</sub> showed anorexia, jaundice, loss of body weight atrophied spleen, and depletion of lymphocytes in germinal epithelium area. Liver revealed hypertrophy of the bile duct epithelium, fibrosis, and adenoma, kidney showed intertubular hemorrhages and atrophy of the glomeruli [58]. A great review exploring the effects of aflatoxins on swine reproduction was written by Kanora and Maes [59].

Stojanac et al. reported acute intoxication in a commercial farm [60]. From Piglets of 21–23 days old, died in 7 days, researchers found 960  $\mu\text{g kg}^{-1}$  of AFB<sub>1</sub> in the compound feed and 870  $\mu\text{g kg}^{-1}$  in sow's milk. After removal of the contaminated feed, the number of deaths began to reduce; the clinical symptoms were apathy, depression, cachexia, move reluctance, and death.

Finally, Azevedo demonstrated that pigs fed  $1.0 \text{ mg AFB}_1 \text{ kg}^{-1}$  feed for 21 days had reduced growth performance associated with altered hepatic gene expression (specifically, cytochrome P450-2A19/CYP2A19 and glutathione S-transferase theta 1/GSTT1 [61]). Furthermore, the authors concluded that supplementation of  $100 \text{ mg curcumin kg}^{-1}$  to diets containing  $\text{AFB}_1$  had a protective effect on changes in gene expression in liver of pigs.

### 3.2. Effects on ruminants

Ruminants are more resistant to the mycotoxins than non-ruminants animals because the rumen microbiota is capable of degrading toxins. However, aflatoxins are only partly degraded by ruminal flora resulting in a secondary toxic and carcinogenic metabolite called aflatoxicol.

In the case of cattle, sheep, goats, and deer, aflatoxins consumption cause reproductive problems, immune suppression, decrease on milk, beef or wool yield, and reduced feed utilization.

Aflatoxins have been shown reduced feed efficiency in cattle; growth can be altered when ruminants consume contaminated feed for extended periods of time.  $\text{AFB}_1$  ( $600 \mu\text{g kg}^{-1}$ ) was shown to depress feed efficiency and rate of gain in steers [56]. It has been attributed to compromise ruminal function by reducing cellulose digestion, volatile fatty acids production, and rumen motility. Acute exposure to aflatoxins causes inappetence and lethargy [62].

Aflatoxin levels between  $100$  and  $1\,000 \mu\text{g kg}^{-1}$  within the diet, cause a decrease in rumen motility, feed efficiency, growth inhibition, and an increase in liver and kidney weight. In lactating dairy cows, researchers report milk production decrease and reduced reproduction efficiency [5]. Embryotoxicity has been reported in animals consuming low dietary concentrations of mycotoxins [56].

In cattle, aflatoxins affect the immune system function by many mechanisms such as inhibition of lymphocyte blastogenesis;  $\text{AFB}_1$  suppress mitogen-induced stimulation of peripheral lymphocytes. Chronic exposure can interfere with vaccine-induced immunity [62].

Aflatoxins affect the milk quality. Cows metabolize  $\text{AFB}_1$  to form the monohydroxy derivative, aflatoxin  $\text{M}_1$  ( $\text{AFM}_1$ ), which is secreted into the cow's milk.  $\text{AFM}_1$  is a potential human carcinogen very resistant to thermal treatments such as pasteurization and freezing. The European Commission Regulation 1881/2006 sets a maximum limit of  $0.05 \mu\text{g kg}^{-1}$  for  $\text{AFM}_1$  in raw milk, heat-treated milk, and milk for the manufacture of milk-based products (EC 2006). Nevertheless, higher levels have been found [63], for example, Škrbić et al. detected the maximum  $\text{AFM}_1$  level of  $1.44 \mu\text{g kg}^{-1}$  with a mean value of  $0.30 \mu\text{g kg}^{-1}$  in commercial milk samples in Serbia [64].

In sheep, high levels of aflatoxins resulted in hepatotoxicosis, nephritic lesions, and mineral metabolism alterations. In lambs,  $2.5 \text{ mg kg}^{-1} \text{ AFB}_1/\text{diet}$  have been reported low feed intake, weight gain, and altered blood parameters [5].

### 3.3. Effects on poultry

Aflatoxin  $\text{B}_1$  has a high range of effects in poultry including acute hepatic toxicity, teratogenicity, carcinogenicity, mutagenicity, hematological problems [65], and immunosuppression.

Poultry is sensitive to low levels of AFB<sub>1</sub>, in order of sensitivity: ducks > turkeys > Japanese quail (*Coturnix japonica*) > chickens [45].

Exposure to aflatoxins has been demonstrated to suppress the immune response in poultry. Both, Rawal et al. and Xi Peng et al. have reported impaired T cell production, decreased phagocytosis and apoptosis in thymus, and bursa of fabricius and spleen [66, 67]. Kumar and Balachandran reported spleen lymphoid and erythroid depletion, enlargement, pallor or yellowish livers, crop and proventricular changes, enlarge, pale and congested kidneys in broiler fed with 1 mg kg<sup>-1</sup> AFB<sub>1</sub> [68].

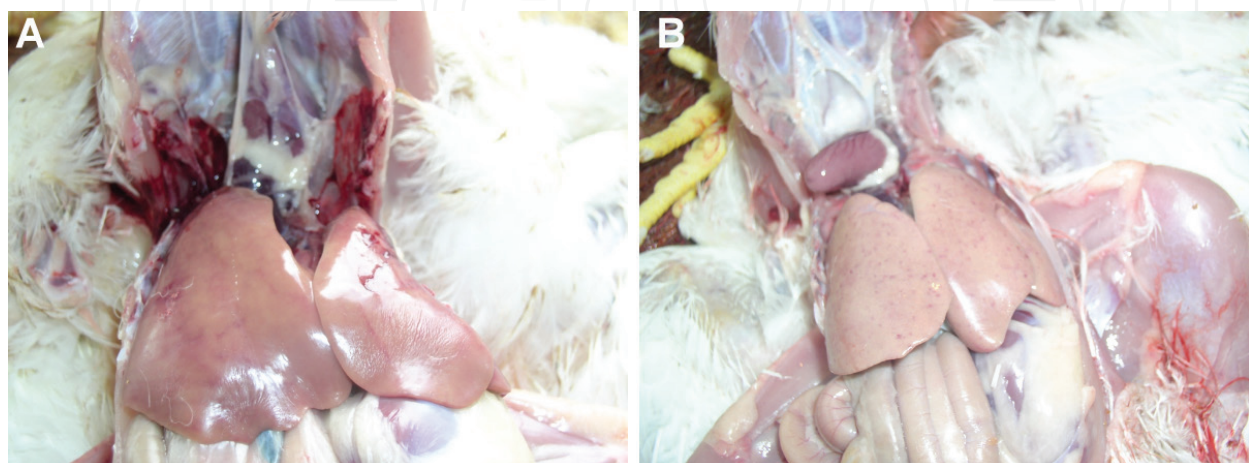
Aflatoxins exposition could be a serious risk to animal health, increasing susceptibility to infections, or reducing vaccination efficacy. Epidemiological data indicate a high correlation between outbreaks of Newcastle disease and AF contamination of broiler rations [69].

Changed serum biochemical parameters, impaired hepatic antioxidant functions, and severe lesions in hepatic tissues were found by Yang et al. in broilers fed with 36.9–95.2 µg kg<sup>-1</sup> AFB<sub>1</sub> [70]. They also observed focal necrosis of hepatocytes, biliary hyperplasia, Kupffer cell hypertrophy, microvesicular fatty degeneration, and apoptosis.

Gross findings in broilers, include paralysis and lying down could be observed, the growth of affected birds is retarded. Additional findings include the yellowish to a yellow-earth color of the liver, the multiple hemorrhages, and a characteristic reticular appearance of the capsular surface. In severe intoxications, the kidneys are enlarged and filled with urates.

Our data also demonstrate abnormal fatty tissue accumulation and hepatic lesions including a suggestive increase in liver size, with the loss of usual color (dark brown), pallor, with visible areas of hemorrhage primarily on the left lobule without gallbladder distension (**Figure 3A**), when chickens were subjected to feeds contaminated with aflatoxin. On the other hand, chicks that were fed with an aflatoxin/T-2 toxin diet exhibited a reduced liver size, greater hepatic paleness, and nodular appearance, without bleeding, cholestatic pattern, or gallbladder distension (**Figure 3B**).

Clinical symptoms seen in poultry are diverse. Hussain et al. reported experimental birds intoxicated with 400–800 µg kg<sup>-1</sup> AFB<sub>1</sub> showed depression, ruffled feathers, watery feces, decrease in water and feed consumption, and nervous signs as torticollis and mortality [71].



**Figure 3.** Chicken liver lesions when subjected to (A) 50 µg kg<sup>-1</sup> aflatoxin diet and (B) 50 µg kg<sup>-1</sup> aflatoxin plus T-2 toxin diet.

Trebak et al. reported listlessness, anorexia [72]; other symptoms include poor feed utilization, stunted growth, decrease weight gain [73, 74]; reduced egg weight and production. High levels of aflatoxins in broilers and turkeys cause hepatomegaly, fatty degeneration, fatty liver, bile conduct proliferation, periportal fibrosis, renal petechiations, tubular nephrosis, interstitial nephritis, and splenic atrophy [67, 75].

Aflatoxins may cause blood coagulations disorders in broilers characterized by extensive hemorrhagic lesions in the stomach, heart, intestines, lungs, kidneys, and muscles resulting in death. Lesions are causal for condemnations in a slaughterhouse. Prothrombin time (PT) is an indicator of aflatoxin toxicity in chickens, the elongation of which is directly proportional to aflatoxin dose and exposure time. PT is an indicator of the activity of blood coagulations factor V, VII, IX, X, prothrombin, and fibrinogen can serve to diagnose liver lesions in poultry [76].

AFB<sub>1</sub> also affect laying hens; losses are pronounced regarding reduced egg production and egg quality as a result of contamination with aflatoxin residues in eggs and muscles. Feed to egg AFB<sub>1</sub> transmission ratio is approximately 5 000:1 [74]. A substantial percentage of the egg samples (28%) showed AFB<sub>1</sub> levels ( $0.79 \pm 0.45 \mu\text{g kg}^{-1}$ ) in commercial eggs [77]. Several authors, reported excretion of aflatoxin B<sub>1</sub> residues in hen's eggs might occur at relatively low concentrations under long-term exposure of laying hens to AFB<sub>1</sub> at different levels up to  $50 \mu\text{g kg}^{-1}$  in a naturally contaminated feed [78–80]. Interestingly, even though Lee et al. found the prevalence for mycotoxins to range from 85–100% in Korean poultry feed samples (n = 20), but they failed to find contaminated egg samples (n = 275) aflatoxins, ochratoxins, or zearalenone [81]. Thermal processing was not useful for detoxification of AFB<sub>1</sub> in eggs [79, 82]. Some researchers have found a significant decrease in feed consumption, egg production, egg weight, shell weight, shell thickness, and feed conversion ratio value in laying hens fed with  $15 \mu\text{g kg}^{-1}$  of AFB<sub>1</sub> [78, 79]. Aflatoxins disrupt the hypothalamic regulation of neuropeptides involved in feeding behavior and contribute to the lower body weight and decreased weight gain [72]. Aflatoxins in the feed of laying hens may cause a relevant lesion in liver, kidneys, heart, and ovaries. The ovaries show follicular atresia, which has a detrimental effect on egg production [79].

Effects of AFB<sub>1</sub> on the absorption of nutrients have had variable results. Mycotoxins can compromise different functions of the gastrointestinal tract such as decreased surface area available for nutrient absorption, modulation of nutrient transporters, loss of barrier function, and facilitating persistence of intestinal pathogens inflammation [83]. However, it is still unclear how the intestinal lesions affect growth and feed efficiency in poultry.

Kalpana et al. found enrofloxacin, and ciprofloxacin residues in liver, kidney, skin, and fat persisted for 10 days in mycotoxin-exposed broiler chickens, whereas it was detectable only in the liver of unexposed broiler chickens, indicating that subchronic AFB<sub>1</sub> exposure markedly influences the residue levels of enrofloxacin in tissues of broiler chickens [84].

Finally, in an interesting report, Iheanacho tested the cytotoxic effect of cattle and poultry aflatoxin-contaminated compound feed extracts on human lymphocytes [85]. The authors observed that cell viability significantly decreased upon contact with feed extracts, especially those from poultry feed, after just 24 hours of exposure, demonstrating that a direct link may be found between human toxicity and feed.



### 3.4. Effects on other species

Marine animals could be exposed to AFB<sub>1</sub> contamination through feed chain [86]. The carcinogenic effect of AFB<sub>1</sub> has been studied in fishes such as salmonids, rainbow trout, channel catfish, tilapia, guppy, and Nile tilapia. Consequences of mycotoxin toxicity in fish do not differ from other animal species. Effects are directly related to losses in production, reduced weight gain, feed conversion, and immune impairment. Kidney, liver, and muscles lesions and residues are found in different species of fish [87].

Cagauan et al. found varying levels of aflatoxin contamination did not significantly affect the final average length, weight, and gain in weight of Nile tilapia; aflatoxin negatively influenced percent survival of fingerlings [88]. External manifestations in fish were eye opacity leading to cataract and blindness, lesions on the body surface, fin and tail rot, yellowing of the body surface, abnormal swimming, feeble and stationary on one place, and reduced appetite. In common carp fingerling (*Cyprinus carpio*) levels of 50 and 100 µg kg<sup>-1</sup> of aflatoxins in the feed affected growth and accumulate in fish tissues [89]. Interestingly, at least two studies have suggested that mycotoxins, such as AFs, can be present in seafood if fish were exposed to mycotoxin-contaminated feed [87, 90].

In horses, AFB<sub>1</sub> in the contaminated feed (58.4 µg kg<sup>-1</sup>) cause jaundice, depression, lameness, anorexia, and death. Ponies have shown damage to the skeletal muscles and heart. Post-mortem lesions show enlarged livers, kidney damage, and bile duct hyperplasia [56]. An excellent review regarding equine health implications of the presence of aflatoxin in feed has been essayed by Caloni and Cortinovia [91].

Mycotoxins on companion animals could be severe and can lead to death. AFB<sub>1</sub> in dogs cause hepatitis and severe depression, anorexia, and weakness. Aflatoxins and other mycotoxins have been found in the ingredients and final products of pet food. Gazzotti et al. found aflatoxins contamination in 88% of the dog food samples, showing concentrations of 5 g kg<sup>-1</sup> [92]. Dog food contaminated with aflatoxins is of particular concern due to the bond companionship animals, or pets usually share with their owners. Frehse et al. not only found a high prevalence of aflatoxins in the commercial feed but also found that of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub> associated positively with mammary tumor growth in female dogs and that neutering was a protective factor for mammary cancer [93].

## 4. Control and management approaches

Mycotoxins are toxic metabolites that can contaminate various crops before or after harvesting. Aflatoxins are a problem also during storage, transport, processing, and handling steps such as manufacturing.

Prevention measurements are focused on the minimization of crop contamination before harvesting (plant breeding and good agronomic practices) and during storage or postharvest (detoxification). Several methods of prevention and control are available to reduce the contamination with aflatoxins. However, mycotoxin contamination of food and feed is unavoidable [94]

mainly because they are ubiquitous nature and current standards are based on regulating the product, not the process. Available approaches are focused on minimizing and mitigating not to eliminate the contamination of both, fungus species and mycotoxins. None of the following methods reduces contamination in high-polluted feed ingredients and foods.

#### **4.1. Pre- and postharvest feed and feed ingredients aflatoxin management: GMP and HACCP practices**

Pre-harvest management of aflatoxins in animal feeds requires an approach based on good agricultural practices by the producer, appropriate legislations and regulation enforcement, constant monitoring of aflatoxins in feeds and foods, and adequate management of contaminated feeds.

Agronomic practices have been shown to have a substantial effect on toxin contamination of crops. The primary strategy should be to prevent mycotoxin production by reducing mold proliferation during cultivation and storage. Practices such as selection of seeds and planting of more resistant varieties of cereals; healthy and vigorous plants capable of withstanding pest attack are required. Molecular techniques are now available as a possible strategy to select varieties on their ability to resist mold attack [95]. Ostrý et al. described that Bt corn showed significantly lower concentrations of aflatoxins than non-Bt corn hybrids [96].

Crop residues are often the primary inocula of mycotoxigenic fungi; removal of agricultural waste is effective in preventing the contamination of follow-on crops [97]. Furthermore, selection of harvest seasons could be a critical approach, showing date partly determine the flowering time, if it coincides with spore release, more frequent and more severe attacks are likely. Early harvesting of groundnuts resulted in lower aflatoxin levels and the higher gross return of 27% than in delayed harvesting [98]. Crop planting should be timed to avoid elevated temperatures and drought stress during the period of seed development and maturation [99].

Other practices such as weed control, crop rotation, plowing, avoiding high plant densities and correct fertilization limits mold contamination and mycotoxin production. Appropriate use of pesticides during the manufacturing process could help in minimizing the fungal infection or insect infestations of crops [56]. Insects can act as fungal spore vectors and attack the grain of external teguments of kernel facilitating colonization of mycotoxin-producing fungi [97]. Dorner and Cole reported soil treatment with non-toxic strains of *Aspergillus* and use of competitive exclusion using bacteria and fungal strains of *Trichoderma* [100] had a beneficial carry-over effect of reducing aflatoxin contamination in crops.

Containers (e.g., wagons and trucks) to be used for collecting and transporting the harvested grain from the field to drying facilities, and, thereafter, to storage facilities should be clean, dry, and free of insects, birds, rodents, and visible fungal growth before use and reuse [99].

Reduction of grain damage before and during storage is important to avoid fungal invasion. Cereals should be dried in such a manner that damage to the grain is minimized and moisture levels are lower than those required to support mold growth during storage [99, 101]. Mixing grains and a long-time storage should be avoided. Grain damaged by mold should be burnt or buried [101].

Quality check of grain and installation integrity before storage and adequate storage conditions (temperature, humidity, moisture, and insect control) are required and must be monitored. Grains should be stored in less than 15 g/100 g of moisture content, at low temperatures and a low oxygen concentration ( $< 1$  mL/100 mL). In tropical and subtropical conditions, grains are more prone to contamination than temperate regions due to favorable humidity and temperature levels for mold growth (10–40°C, pH range of 4–8 and above, 70% relative humidity) [101]. For example, in Turrialba, Cartago, Costa Rica (9°54'00"N 83°41'00"W), reported a mean temperature and relative humidity of  $(22.0 \pm 0.7)^\circ\text{C}$  and  $(87.7 \pm 2.2)\%$ , respectively.

In storage, many insect species can attack the grain and moisture that can accumulate from their activities providing ideal conditions for fungal activity and management of insect infestations which is required. Prevention of insect pest is desirable but the intensive use of chemical compounds has resulted in the evolution of resistant populations. Phosphine gas is a common and toxic fumigant used for disinfection of storage grains. Essential oils, application of ozone, and use of diatomaceous earth are alternatives to phosphine gas to control insect pest in storage grains.

The addition of antifungal agents, preservatives, antioxidants, essential oils, and controlled atmospheres, may help to reduce fungal growth during storage. Antioxidants such as selenium, vitamins A, C, and E, ethoxyquin, and butylated hydroxytoluene [102] have been recognized as anti-aflatoxic agents. Food components (fructose, phenolic compounds, coumarins, and chlorophyll) and food additives (piperine, aspartame, cyproheptadine, and allyl sulfides) have shown toxicity reduction of several mycotoxins [103]. Weak acids are used in animal food and feed to prevent fungal spoilage; the most common are propionic, benzoic, and sorbic acid.

Some essential oils have fungicidal actions such as carvacol,  $\alpha$ -p-cymene, terpinolene, anethole, and eugenol. Esper et al. described a considerable AFB<sub>1</sub> reduction in corn, and their efficacy depended mainly on the essential oil concentrations and substrate water activity conditions, concentration, and incubation periods [104]. Hence, essential oils can find a practical and safe application in toxin control [105].

Modified atmospheres (low O<sub>2</sub> and high CO<sub>2</sub> concentrations) are used for fungal growth monitoring and mycotoxin production in stored grains. Silo-bags are also used. They are water-proof and have some level of gas-tightness (O<sub>2</sub> and CO<sub>2</sub>). The use of ozone as a strategy to control toxigenic fungi and mycotoxins production needs further evaluations [102].

Hazard analysis critical control point (HACCP) system has been increasingly and successfully applied by the grain and feed industry to prevent and control risks associated with potential contamination with toxins [106]. Mycotoxins can be classified as a biological or a chemical hazard [102]; they fit in an HACCP program at appropriate critical points, and their critical limits must be identified. For example, a critical control point could be at the end of the drying process, and one critical limit would be the water content/water activity [99]. Also, FAO recommends the application of an HACCP program for the systematic control of mycotoxins through the entire food chain from field to consumption including all pre-harvest, harvest, and postharvest stages in the production of animal feed and animal feed ingredients. Additionally, FAO has published a manual to make easier the application of this mycotoxin control program (<http://www.fao.org/docrep/005/Y1390S/Y1390S00.HTM>).

The efficient and prompt drying of corn for medium- and long-term storage in hygienic silos free of insect pest and fungal populations and accurate and regular moisture content, water activity ( $a_w$ ), fungal growth, insect presence, bacterial level, the percentage of grain damage, storage time, storage temperature, and humidity measurements must be considered in an HACCP program [101, 102]. Pre- and postharvest measures are paramount to avoid the risk of contamination in both feeds and foods; new trends in the decontamination of aflatoxins [107] should be considered as complete absence of such toxins which is extremely difficult. Lastly, as a case study, we highlight the work of Kamala et al. [108]. The authors examined three agro-ecological zones of Tanzania and determined that local postharvest management practices such as drying corn on a raised platform, sorting (damaged, discolored, and molded grains) and application of synthetic insecticides during storage, associated with less contamination of corn with aflatoxins and fumonisins.

#### 4.2. Decontamination of mycotoxin-contaminated feed

There are different approaches to decontaminate or detoxify a feed or food commodity containing mycotoxins, among them the use of mycotoxin binders in the feed, enzymatic, or microbial detoxification. Some chemical substances have been assayed to reduce aflatoxins, especially ammonia. However, chemical detoxification is expensive and though permitted in some countries, is not so in Europe. Hence, the most common postharvest approach in the feed industry is the inclusion of sorbent materials in the feed to obtain selective removal of toxins by sorption during passage through the gastrointestinal tract [97]. The mycotoxin binders are also called adsorbents, mycotoxin binders, sequestrants, interceptor molecules, trapping agents, or enterosorbents. There are inorganic sorbents principally clay minerals and organic sorbents of microbial origin [42]. In some cases, they have the ability to bind mycotoxins and reduce their absorption across the gastrointestinal tract [109].

Decontamination process should include inactive mycotoxins, generate no toxic products, and guarantee no modification of nutritional properties of the feed or food. The properties of adsorbents are important in the evaluation of their efficacy: physical structure, effectiveness at different gastrointestinal pH levels (acidic and neutral), total charge, distribution, pore size, and surface accessibility should be considered. However, the diversity of mycotoxins chemical structures makes difficult that a single method can decontaminate an animal feed [42].

Mycotoxin characteristics such as polarity, solubility, molecular size, shape, charge distribution, and dissociation constants must be evaluated. Sorbents have been tested using *in vitro* and *in vivo* systems, *in vitro* studies are very common and *in vivo* tests [97] are used to find performance responses or biological markers such as tissue residues or changes in biochemical parameters to determine the effectiveness of binders. A suitable adsorbent or binder should have an unyielding bonding, so no washing or interactions in the digestive tract desorb the bound mycotoxins. Binder use and efficacy should be verified.

Silicate binders are divided into subclasses according to their structure; one group is the phyllosilicate family characterized by the sheet-type framework [97]. Hydrated sodium calcium aluminosilicates (HSCASs) are the most reported; they adsorb aflatoxin selectively during the digestive process, and it involves the formation of a complex by the  $\beta$ -keto-lactone or lactone system.

Other silicates studied are bentonites, zeolites, and clinoptilolites. Other mineral adsorbents include synthetic polymers such as cholestyramine and polyvinylpyrrolidone, indigestible dietary fibers also have absorbance effect. Mineral binders are efficacious against aflatoxins, but they are not very specific and can absorb other molecules such vitamins and others nutrients [110].

Organic substances such as humic acids have the ability to adhere mycotoxins, yeast, and yeast extracts are also able to reduce the aflatoxin effect. Parietal structures of some lactic acid bacteria have the potential to bind mycotoxins; the adsorption is reversible and could be performed with living or dead bacteria. Other biological materials such as fungal conidia have binder effect against AF, zearalenone, and ochratoxin A.

#### 4.2.1. Efficiency of aflatoxin sorbents

The inclusion of different types of adsorbents especially clay minerals has been widely used in the feed and farm industry to counteract the mycotoxins toxic effects in animals [42]. The easy management and low inclusion requirement in feed make the use of adsorbents a standard practice. There are some studies about the protective effect of these sorbents in different animal species especially food-producing animals such as pig, poultry, and cattle using different mycotoxins and different concentrations and testing the various health and productivity parameters. These trials have shown variable results with more or less successful depending on the adsorbent, the mycotoxin, the species, and the parameters tested.

Mitchell et al. have reported that calcium dioctahedral smectite clay has the capability to adsorb mycotoxins in the gastrointestinal tract decreasing toxin bioavailability reducing biomarkers of exposure for AFB<sub>1</sub> as well as FB<sub>1</sub> [111]. Furthermore, other studies have reported the ability of “dioctahedral smectite” clay surfaces to strongly adsorb aflatoxins [112]. This ability is not associated with other clay groups such as kaolinites, attapulgites, zeolites, mica, alumina, and sand [42].

Among the sorbents used by the farm and feed industry are smectite clays, zeolites, kaolinite, mica, silica, and charcoal. Smectite or zeolite minerals with natural or synthetic surfactants giving hydrophobic organoclays or organozeolites are also used [113–115]. There are also sorbents of biological nature such as chlorophyllins, yeast products, lactic acid bacteria, plant extracts, and algae [42].

The aflatoxins adsorbents should be carefully tested through *in vitro* and *in vivo* studies, and they should fulfill some safety and economic aspects such as stable and high adsorption capability with different mycotoxins, insignificant interactions with vitamins, iron, and zinc, low levels of metals dioxins/furans and other hazardous substances. The European Food Safety Authority (EFSA) has published guidelines pointing out the characteristics that the adsorbents should fulfill [116].

Dos Anjos et al. investigated the efficacy of three different aflatoxins adsorbents: bentonite clay, diatomaceous earth, and turmeric powder in broiler chicks feeding aflatoxins contaminated diets [117]. They found that birds fed with turmeric (without aflatoxins) presented lower body weight gain than control animals. The birds fed with AFB<sub>1</sub> and adsorbent bentonite clay did not experiment the decrease of feed intake and feed gain occurred in the birds

fed with AFB<sub>1</sub>. Birds fed with diet containing AFB<sub>1</sub>, diatomaceous, and tumeric had poorer growth performance than those fed on AFB<sub>1</sub> alone. The toxicity effects and lesions in liver were not counteracted by any of the adsorbent treatments [117].

Commercial products based on this rationale are available, for example, Alltech® Mycosorb A<sup>+</sup>. Sun demonstrated that diets with Mycosorb A<sup>+</sup> (2 g kg<sup>-1</sup>) could improve growth performance in swine by increasing average daily gain and average daily feed intake, whereas low-level aflatoxin (20 µg kg<sup>-1</sup>) had minor effects on hematology without affecting growth performance [118]. On another hand, aluminosilicates, zeolites, and other chemisorptive agents have been assayed against aflatoxins with relative success. In a recent publication, Wongtangtintan et al. demonstrated that thai bentonite exhibited an excellent binding capacity toward AFB<sub>1</sub> surpassing commercial bentonite and activated charcoal *in vitro* [119]. Furthermore, the authors suggest that the adsorption behavior of AFB<sub>1</sub> on these toxin binders represented multilayer/multiple site adsorption on the binders' surfaces. An excellent review of experimental trials demonstrated different detoxification approaches in poultry feed had been written by Oguz et al. [65]. In broilers, a study performed by Denli et al. demonstrated that supplementation of AflaDetox® significantly ameliorated the toxic effects of AFB<sub>1</sub>. The authors suggest that the addition of AflaDetox (1, 2, and 5 g kg<sup>-1</sup> of feed) to diets containing AFB<sub>1</sub> significantly improved performance, counteracted the serum biochemical and histopathological changes, reduced the relative weight of liver, and also appeared to be effective in reducing the relative spleen weight [120]. Some data supporting the effectiveness of adsorbents must be considered with caution as in some cases, chemisorbent developers have participated, to some degree, in the research hence creating an apparent conflict of interest (see, e.g., Ref. [120]).

A study carry out by Neeff evaluated the efficacy of a HSCAS reducing aflatoxin residue in tissues of broiler chicks. The author found that with adding this adsorbent in the diet the concentration of aflatoxins residues in liver was lower than in birds consuming a diet contaminated with AFB<sub>1</sub> without HSCAS [121]. Despite this, as in the study carried out by Dos Anjos et al. [117], this adsorbent could not avoid the lesions in the liver associated with aflatoxicosis in broilers [121]. On the other hand, Fowler et al. did observe an improvement in broilers incorporating 0.2 g/100 g calcium bentonite clay additive (TX4) [122]. The additive effectively reduced the accumulation of AFB<sub>1</sub> in the liver, improving livability in birds fed aflatoxin.

In a previously study carry out by our research group, we evaluate three different mycotoxin adsorbents (HSCAS) in broiler chicken feed aflatoxins contaminated diet. We found little ameliorative effect of some parameters such as creatinine and alanine aminotransferase (ALT) in broilers fed with contaminated diet and the adsorbents compared with broilers fed only aflatoxins diets. However, we found a significant higher liver weight in broilers getting AFB<sub>1</sub> and two of the tested adsorbents in comparison with broilers getting only AFB<sub>1</sub> [123]. From the feed technology standpoint, Maki et al. demonstrated that 6 g calcium montmorillonite clay (Novasil Plus, NSP)/kg feed, can significantly decrease AFM<sub>1</sub> concentrations (up to 55% reduction) in milk without affecting dry matter intake (DMI), milk yield, milk composition, vitamin A, or riboflavin concentrations [124]. Similarly, Mugerva et al. demonstrated that 1 g/100 g of calcium bentonite and charcoal reduced AFM<sub>1</sub> carry-over in goats fed with contaminated feed while DMI and daily milk yield were not altered with treatment [125].

#### 4.2.2. Novel approaches for tackling aflatoxin contamination

Since sorbents have demonstrated a limited capability in toxin management and preventive measurements are difficult to apply, new tactics to control aflatoxins are continually being developed. For example, Wee et al. suggested that use of zinc chelators (e.g., *N,N,N',N'*-tetrakis(2-pyridylmethyl) ethane-1,2-diamine) has the potential of diminishing the capacity of *A. parasiticus* to produce toxins [126]. In fact, they observed significant inhibition of aflatoxin production but no detectable changes gene expression (i.e., *ver1* and *aflR*). Furthermore, the authors demonstrated the efficacy of this approach in peanut and sunflower seeds. Weaver et al. used clay and yeast cultures conjointly to improve amelioration in aflatoxin and deoxynivalenol-contaminated swine feed [127]. Interestingly, Das et al. demonstrated that *Pleurotus ostreatus*, a fungus that can grow on different agronomic wastes, can synthesize several ligninolytic enzymes which are capable of degrading compounds including AFB<sub>1</sub> [128]. Additionally, the authors demonstrated that AFB<sub>1</sub> degradation occurs during co-cultivation of *A. flavus* and *P. ostreatus* in rice straw, a common feed for cattle. Similarly, Lee et al. also demonstrated *Aspergillus oryzae* (a microorganism used as a fermentation starter in Meju) capability for detoxification of AFB<sub>1</sub> [129]. Villers [130] detailed field experience governing the exponential growth of aflatoxins during prolonged postharvest storage of grains in tropical countries. In this case, the authors focuses on modern, safe storage methods to ameliorate mold development and subsequent aflatoxin production using UltraHermetic™ structures that generate an atmosphere incompatible with insect and microorganisms' survival, without further use of other additives. Bovo et al. evaluated the capacity of a beer fermentation residue (BFR) containing *Saccharomyces cerevisiae* cells to bind AFB<sub>1</sub> and counteract its toxic effects on performance, serum biochemistry, and histology of broilers. Feed intake, body weight gain and concentrations of albumin, total protein, and globulin increased in broilers fed aflatoxins contaminated diet with BFR in comparison with the broilers that only receive AFB<sub>1</sub>. The BFR reduced the severity of histological changes in the liver and kidney caused by AFB<sub>1</sub> but not the effect on kidneys and liver weight [131]. Pizzolito et al. demonstrated a protective capacity of *S. cerevisiae* specifically against aflatoxins in poultry when added to feed and water [132].

Recently, our research group found that the milk proteins casein and the milk whey protein are capable to sequester aflatoxins M<sub>1</sub> *in vitro*; this bind capability should be further investigated and could be used in further AFM<sub>1</sub> detoxification intervention in the dairy industry [133].

Yin et al. demonstrated, using poultry feed as a substrate, that carvacol and *trans*-cinnamaldehyde inhibit *A. flavus* and *A. parasiticus* growth and downregulates aflatoxin synthesis genes (*aflC*, *nor1*, *norA*, and *ver1*). Similarly, Nerilo and et al. demonstrated that *Zingiber officinale* fully inhibited aflatoxin production by *A. flavus* at a concentration of 15 µg mL<sup>-1</sup> [134].

Furthermore, there are other detoxification approaches based on the transformation of the mycotoxin compounds using microorganism or enzymes. Nowadays, approaches in ameliorating toxin burden have relied heavily on biological methods. An excellent review on the subject was made recently by Ji et al. [135]. An additional point regarding detoxification relies on the fact that they must demonstrate their binding capacity both *in vitro* and *in vivo* through a report on this subject with the most recent advance written by Wielogórska et al. [136]. Jiang

et al. demonstrated the efficacy of Bamboo charcoal as an agent capable of ameliorating AFB<sub>1</sub> on an *in vitro* rumen fermentation of a hay-rich feed mixture, the authors assayed 1.0 µg mL<sup>-1</sup> and compared the effectiveness of this alternative to that of smectite [137]. A novel approach was introduced by Zhao et al. who detoxified peanut meal using solid state fermentation and *Zygosaccharomyces rouxii* from fermented soy paste [138]. The authors demonstrated nonviable cell binding and biotransformation of AFB<sub>1</sub> in which reduction was monitored by LC/MS. A recent pertinent study by Shar suggested that banana peel (*Musa* sp.) may be used as bioadsorbent for AFs and ochratoxin A *in vitro* [139]. Using thermodynamic properties of adsorption, the authors demonstrated that sorption was not affected by low pH, simulating conditions of the gastrointestinal tract, and, even, suggested to incorporate this by-product in animal feeds as economic sorbent.

Finally, evidence suggests that the oxidative stress is a key factor in aflatoxin-related pathology, specifically the role of glutathione [140]. In fact, Jardon-Xicotencatl et al. using neutral electrolyzed oxidizing water demonstrated that lipid peroxidation and oxidative damage (based in glutathione modulation) are reduced when aflatoxin-contaminated corn is treated [141]. Hence, animal antioxidative balance is paramount to counter, detoxify, and ameliorate aflatoxin burden. Then, from the nutritional standpoint, there is room to improve diets and feed formulations using effective antioxidants, which are usually overlooked.

### 4.3. Aflatoxins and climate change

We already established that aflatoxin production is dependent on multiple environmental factors including temperature and humidity. Hence, climate change intrinsically forces a new dynamic in those naturally produced contaminants. Countries in the tropical fringe, such as Costa Rica, are experiencing an increase in sparing rains during dry seasons increasing relative humidity and rise in overall temperatures. Countries with more proximity to the poles are projecting unusual weather as well, dependant of the region. For example, in an interesting study carried in Southern Norway by Uhlig et al., the authors found *Aspergillus* metabolites (e.g. sterigmatocystin) in concentrations up to 20 µg kg<sup>-1</sup> [142]. Samples analyzed included barley ( $n = 20$ ), oats ( $n = 28$ ), and wheat ( $n = 28$ ) collected during the wet summer seasons were analyzed using an LC-MS/MS ESI<sup>±</sup>. In this regard, some authors already have stated that aflatoxins are among the foodborne risks most susceptible to climate change [143, 144]. Hence, meteorological data should be collected alongside aflatoxin incidence and levels. Several studies have focused on this particular subject [41, 145]. More recently, Mitchel et al. presented an interesting study case which described corn contamination dynamics influenced by weather patterns [146]. As explained before, corn is rather important feed ingredient. Nestic et al. mentioned that plant physiology is also altered as plants are subjected to different photoperiod and temperature regimes, this applies stress to productive species such as corn [143]. Battilani et al. described climate change as a motor force for emerging feed safety issues and elegantly predicted through climate mathematical model aflatoxin contamination in corn and wheat crops [146]. The authors predicted within the next 100 years a +2°C and +5°C climate change scenario, which converts aflatoxin in corn in a food safety issue. Medina et al. described the interaction among  $a_w$ , temperature and CO<sub>2</sub> and their effect on the relative expression of AF



biosynthetic genes, *A. flavus* growth and aflatoxin production under elevated temperature and drought conditions [147]. The authors concluded that such environmental conditions had limited effect on growth, but significant impact on gene expression (both, structural *aflD* and regulatory *aflR* genes) and significantly arouse the production of AFB<sub>1</sub>. The authors demonstrate these effects *in vitro* and on corn grains.

## 5. Conclusions and perspectives

Conclusive diagnostics regarding aflatoxicosis is difficult, confounding symptoms can cause an animal with aflatoxicosis to be misdiagnosed. In-farm productivity issues caused by toxins can be easily overlooked. On the other hand, farmers may equivocally attribute productivity loss to toxin presence where none is found. Herein we presented several approaches to control toxin in feed production and evidence suggest that GMP, and HACCP should be mandatory as a preventive measure to control aflatoxin contamination. Independently of which countermeasures are selected and applied, they should be pragmatic and implemented in conjunction with those designed for prevention. Changing patterns in weather add hindrance in the prediction of aflatoxigenic fungi colonization and toxin production; hence, countries should increase vigilance and take further preventive and control measures to respond swiftly to an eventual increase in toxin incidence due to regional climate change. Finally, considering the relevance of feed in the food chain safety, countries should implement and improve monitoring programs for aflatoxin in foodstuffs; these programs should contemplate risk management to mitigate the economical and health burden aflatoxin contamination generate.

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## Author details

Andrea Molina Alvarado<sup>1,2</sup>, Rebeca Zamora-Sanabria<sup>1,2</sup> and Fabio Granados-Chinchilla<sup>2\*</sup>

\*Address all correspondence to: [fabio.granados@ucr.ac.cr](mailto:fabio.granados@ucr.ac.cr)

1 Universidad de Costa Rica, Centro de Investigación en Nutrición Animal and Escuela de Zootecnia, San José, Costa Rica

2 Universidad de Costa Rica, Centro de Investigación en Nutrición Animal, San José, Costa Rica

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