Aflatoxins in Feed

Subjects: Biochemistry & Molecular Biology

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Feeding farm animals with aflatoxin-contaminated feed can cause various severe toxic effects, leading to increased susceptibility to infectious diseases and increased mortality, weight loss, poor performance and reduced reproductive capability. Following ingestion of contaminated foodstuffs, aflatoxins are metabolized and biotransformed differently in animals.

mycotoxin aflatoxin toxicity metabolism swine decontamination

1. Introduction

Mycotoxins are toxins produced by certain fungal species. They are classified into five main groups (**Figure 1**), with specific chemical structures, that occur frequently in foods and feeds, i.e., trichothecenes, zearalenone, ochratoxins, fumonisins and aflatoxins. At the same time, fungi that produce mycotoxins are divided into two groups: those that invade before grain harvesting, a group commonly called field fungi, and those that grow only after harvesting, called storage fungi. Among the field fungi, several types of mycotoxin-producing species can be distinguished. The most important are i. *Fusarium graminearum* (deoxynivalenol, nivalenol), normally developed on the field plants; ii. *Fusarium moniliforme* (fumonisins), and sometimes *Aspergillus flavus* (aflatoxin), present in the case of senile or stressed plants; iii. *Penicillium verrucosum* (ochratoxin) and *A. flavus* (aflatoxin) that colonize the plant prior to harvesting, and subsequently predispose the crop to mycotoxin contamination. Mycotoxins are spread in animal feed, cereal crops, vegetables, and animal products. Feeding stuffs for farmed animals are considered as having the highest levels of mycotoxins [1][2][3][4][5][6].

Aflatoxins are a group of secondary metabolites that are produced by several *Aspergillus* species with increased toxicity and carcinogenic potential. Pigs, poultry and cattle are the most important farm animals affected by aflatoxicosis. The most potent toxicant is AFB1 [7].

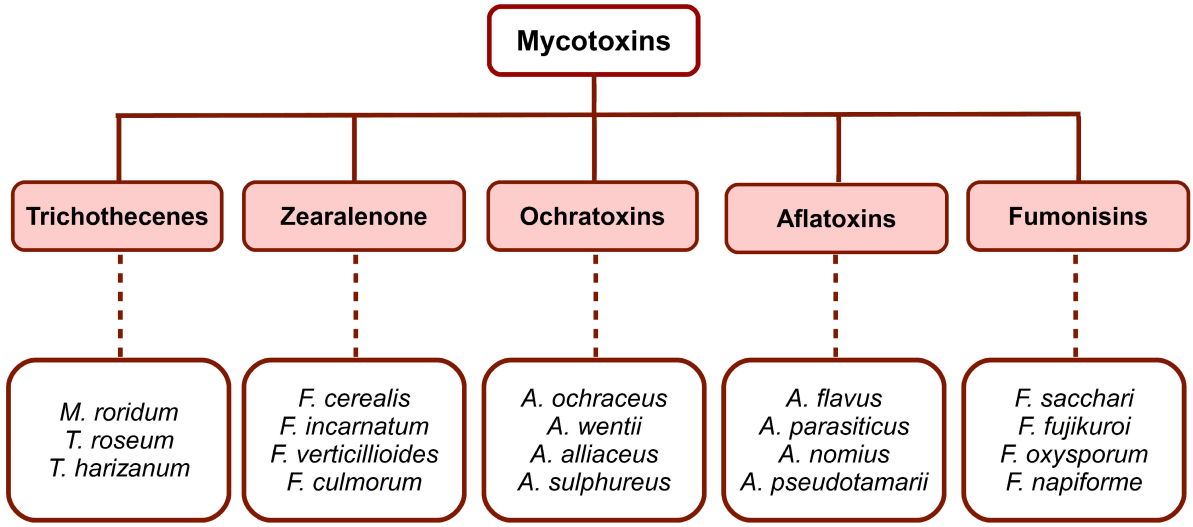

Figure 1. Classification of mycotoxins and the main producing species. Adapted after [8][9][10][11][12][13]. This image was made in OpenOffice Draw software.

Until 1985, the Food and Agriculture Organization reported that approximately 25% of the world's agricultural production is contaminated with mycotoxins [14]. Taking into consideration the predicted climate change in southeastern Europe, increased cereal contamination with AFB1 and OTA is expected [15]. Contamination with aflatoxins is most predominant in the regions of Africa and Asia, due to climatic conditions that favor the development of aflatoxigenic strains in both field and storage conditions [16][17]. The risks of aflatoxin-contaminated feed depend largely on the age and physiologic status of farm animals.

2. Types of Aflatoxins

Mycotoxins are natural compounds of low molecular weight, up to 500 Da; aflatoxins are considered the most toxic, responsible for a significant decline in agriculture. They represent the most abundant groups found in foodstuffs, oilseeds, cereals, and dairy products [6][18]. All types of aflatoxins are derived from fungal species belonging to the genus *Aspergillus* and are considered among the most harmful mycotoxins for both animals and humans [19][20][21][22][23].

Aflatoxins are colorless to pale yellow crystalline substances, freely soluble in moderately polar solvents such as chloroform, methanol, dimethyl sulfoxide, with a water solubility of 10–20 µg/mL. In conditions such as under ultraviolet light in the presence of oxygen, extremes of pH < 3 or pH > 10 and oxidizing agents, aflatoxins are unstable. For example, ammonization at high temperatures results in the opening of the lactone ring, generating the decarboxylation of an aflatoxin molecule, an irreversible reaction. Some important physical and chemical properties of aflatoxins are given in **Table 1** [20][24][25][26][27][28][29].

Currently, over 20 types of aflatoxins are known and among the best known are B1, B2, G1, G2, M1, M2, aflatoxicol and aflatoxin Q1 (**Figure 2**). Some of these forms are derivatives or metabolites of animal metabolism.

For example, aflatoxin M1 and aflatoxin M2 are the metabolites of aflatoxin B1 and aflatoxin B2 which are found in the milk of lactating mammals fed with aflatoxin-contaminated feed [20][29][30].

Table 1. Physical and chemical properties of major aflatoxins. Adapted after [29][31][32][33][34].

Aflatoxin Type	Molecular Formula	Molecular Weight (g /mol)	Melting Point (°C)	Fluorescence	
				λ Excitation (nm)	λ Emission (nm)
B1 [29]	$C_{17}H_{12}O_6$	312	268–269	223	425
B2 [29]	$C_{17}H_{14}O_6$	314	286–289	265	425
G1 [29]	$C_{17}H_{12}O_7$	328	244–246	243	450
G2 [29]	$C_{17}H_{14}O_7$	330	237–240	265	450
M1 [33]	$C_{17}H_{12}O_7$	328	299	365	435
M2 [34]	$C_{17}H_{14}O_7$	330	293	360	450
Aflatoxicol [32]	$C_{17}H_{14}O_6$	314	225	325	425
Aflatoxin Q1 [31]	$C_{17}H_{12}O_7$	328	250	365	466

2.1. Aflatoxins B1 and B2

Aflatoxin B1 (AFB1) is the most potent carcinogenic mycotoxin naturally produced by Aspergillus species such as A. flavus, A. parasiticus, A. nomius, A. bombycis, A. arachidicola, A. minisclerotigenes, A. ochraceoroseus, A. pseudotamarii and A. rambellii, and it exerts harmful effects on humans and animals. The sensitivity degree and toxicity of AFB1 vary significantly between species, due to differences in its biotransformation. Some animals are considered extremely susceptible to AFB1, especially turkeys, rats, pigs, sheep, and dogs, whereas others such as monkeys, mice and chickens are considered resistant. The LD_{50} values for aflatoxin B1 are variable, depending on species and sex, with values ranging from 9 to 60 mg of AFB1 per kg of body weight [20][30][35][36][37][38].

Aflatoxin B2 (AFB2) is a blue-fluorescent, toxic secondary metabolite produced by the same species as AFB1, such as *A. arachidicola, A. flavus, A. minisclerotigenes, A. nomius* and *A. parasiticus*. This metabolite can be synthesized through multiple sequences that begin with a [2+3]-cycloaddition between quinone and 2,3-dihydrofuran [20][39][40][41].

2.2. Aflatoxins G1 and G2

Aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) are toxins produced by species of the common soil fungi, *A. parasiticus, A. nominus, A. bombyccis, A. arachidicola* and *A. flavus*. The presence of AFG1 is associated with

toxicity and hepato-carcinogenicity in human and animal populations, while AFG2 has much lower activity [20][30][42][43].

2.3. Aflatoxins M1 and M2

The aflatoxins M1 (AFM1) and M2 (AFM2) are mammalian bio-conversion products or 4-hydroxy derivatives of AFB1 and AFB2, respectively, produced by *A. flavus* and *A. parasiticus*. After entering the body of humans or animals, AFB1 and AFB2 are metabolized by the hepatic microsomal mixed function oxidase system (cytochrome P450) to a reactive epoxide intermediate, but they can be also hydroxylated to the less harmful aflatoxins M1 and M2. In the case of an animal that ingests feed contaminated with AFB1, a percentage between 0.5% and 5% of the toxin ingested is biotransformed in the liver into AFM1. Milk, cheese, and other dairy products contain residues of AFM1 and AFM2 that should not exceed the limit of 50 ng per kg in Europe, 500 ng per kg in the USA, and 100 ng per kg in Iran [20][23][30][44][45][46][47] for human consumption.

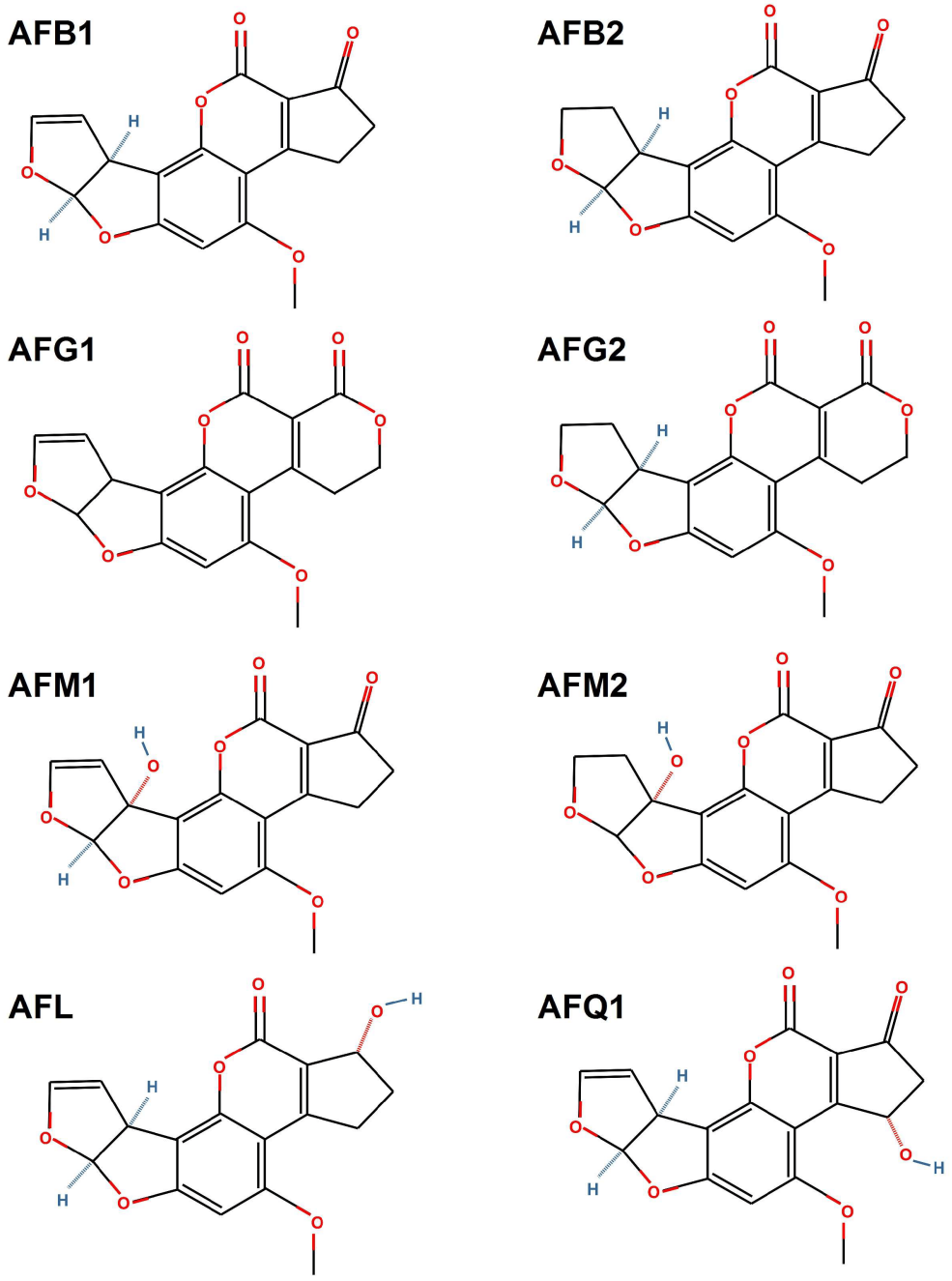

Figure 2. Chemical structures of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1), aflatoxin M2 (AFM2), aflatoxicol (AFL) and aflatoxin Q1 (AFQ1). This image was made in OpenOffice Draw software, v 4.1.9.

2.4. Aflatoxicol

The first report on natural contamination of food with aflatoxicol (AFL) appeared in 1984 [48]. AFL is one of the metabolites of AFB1, formed by the selective reduction of cyclopentanone carbonyl of AFB1, and has two stereoisomers (AFL1 /AFL-A /Ro and AFL2 or AFL-B) which differ by the orientation of the hydroxyl group in the cyclopentene ring. Both AFL forms are produced by the biological reduction catalyzed by enzymes present in fungi, such as: *Tetrahymena pyriformis, Trichoderma viride, Dactylium dendroides, Streptococcus lactis, Absidia repens,*

Mucor griseocyanus, Aspergillus niger, Mucor ambiguus, Tetrahymena pyriformis and *Rhizopus spp*. Although AFL is eighteen times less toxic than AFB1, it was shown that AFL is carcinogenic and a potent frameshift mutagen [32][49][50][51][52].

2.5. Aflatoxin Q1

Aflatoxin Q1 (AFQ1) is a monohydroxylated derivative of AFB1, being one of the major AFB1 metabolites which appear after incubation of microsomal fraction from the mammalian liver with AFB1. The microsomal fraction is rich in CYP3A4 and other CYP450 enzymes which are responsible for the activation of AFB1 into the epoxide form, and for conversion into a less toxic detoxification metabolite, AFQ1. Initially it was found in the urine of rhesus monkeys orally exposed to AFB1. On the other hand, Yourtee et al. [53] showed that AFQ1 might be a major metabolite in the detoxification pathway of the native mycotoxin. AFQ1 is approximately eighteen times less toxic and approximately eighty-three times less mutagenic than AFB1 [30][53][54][55].

3. Aflatoxins' Metabolism: Biochemical, Molecular and Cell Signaling Aspects

After ingestion of contaminated food, aflatoxins are absorbed in the intestine; following their distribution, metabolism and excretion, the liver is the first and main organ affected (**Figure 3**). They also accumulate in muscle. P450 cytochromes play an important role in phase I biotransformation of xenobiotics, especially those belonging to families 1 and 3 [56]. In mammals, the enzymes with the highest levels of protein expression, and involved in the conversion of aflatoxins, are CYP1A2 and CYP3A4. The metabolite resulting from the oxidation reaction can bind to DNA, causing genotoxicity, and proteins generating cytotoxicity. For example, AFB1 binds to guanine residues of nucleic acids, resulting in AFB1 adducts that can lead to transversion of guanine–cytosine (GC) to thymine–adenine (TA) and implicitly to irreversible DNA damage. Binding of AFB1 to proteins is irreversible, the most well-known adduct being ADB1-lysine in albumin. In the first stage of metabolic oxidation in the liver, an epoxy reactive intermediate (e.g., AFB1-8,9-epoxide) is formed or this is hydrolyzed to a less toxic form, AFM1 [57][58].

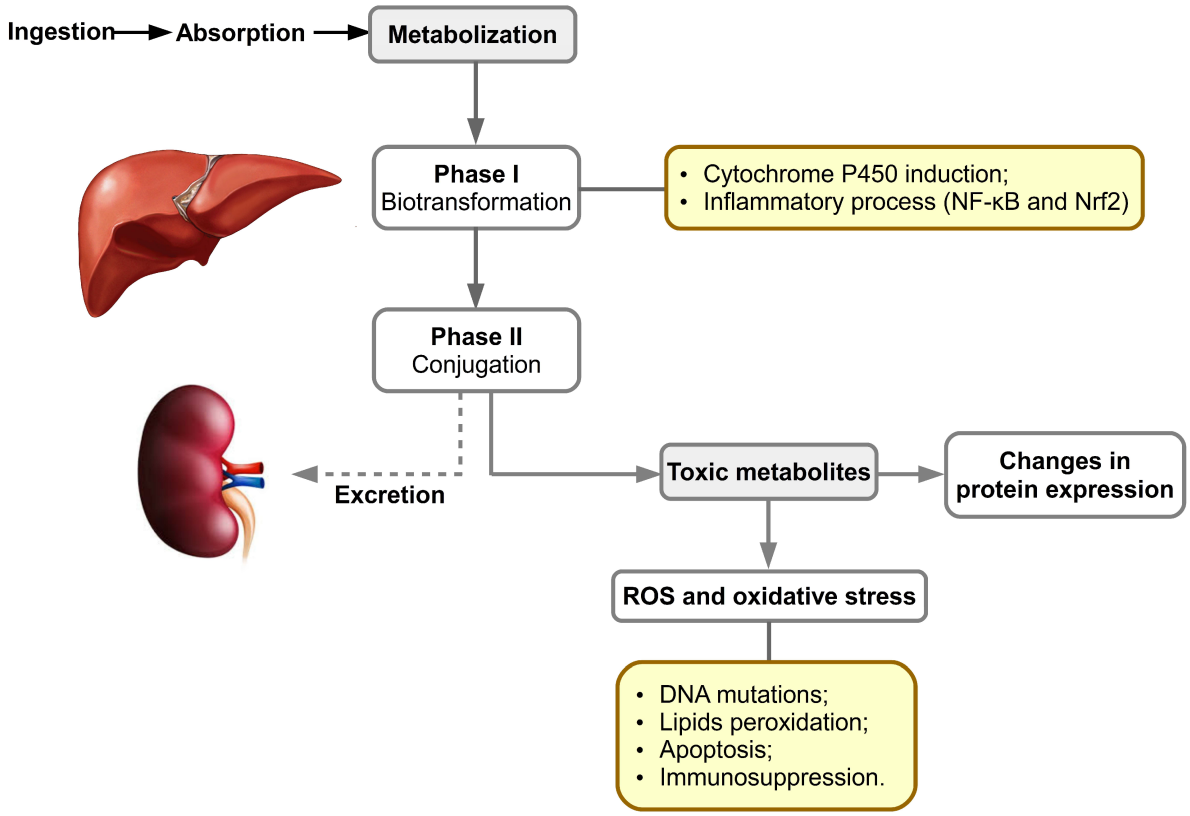

Figure 3. The adverse cellular effects of mycotoxins and their metabolites. Adapted after [56][59][60][61]. This image was made in OpenOffice Draw software, v 4.1.9.

The cytochrome P450 superfamily consists of enzymes involved in xenobiotic metabolism and endogenous compound oxidation; thus, Phase I enzymes catalyze the reactions of hydroxylation, sulphoxidation, epoxidation, N-, O- and S-dealkylation, oxidative aromatic hydroxylation, desulfuration, denitrosation, and dehalogenation aiming for the addition of functional polar group(s). In porcine hepatic tissue, the CYP450 proteins expressed are represented by CYP2A19 (34%), CYP2D25 (25,5%), CYP2C49 (11.2%), CYP2E1 (8.1%), CYP3A39 (8,1%), CYP3A29 (5,8%), CYP2C33 (5%) and CYP1A2 (2.3% of the total liver CYPs, respectively) [62][63][64][65][66][67][68].

Phase II of metabolism implicates conjugation reactions of metabolites previously formed [69] with glucuronic acid and sulfate especially. Subsequently, the epoxide metabolite generated in phase I may be detoxified in phase II by glutathione conjugation, through hydrolysis by an epoxide hydrolase to AFB1-8,9-dihydrodiol, or by reduction to a less toxic metabolite such as AFM1 or AFQ1 [43][70][71][72][73]. The resulting metabolites are excreted through the biliary pathway, followed by the urinary pathway.

By RNA-seq technology it was proved that in vitro exposure of bovine fetal hepatocyte cell line (BFH12) to AFB1 affected the cells` transcriptome. Gap junction protein beta 2 and Follistatin genes—the latter being involved in proliferation and colony expansion of progenitor populations of hepatocytes—as well as those of ornithine decarboxylase and A-Raf proto-oncogene have been upregulated. Instead, genes that codify for tumor suppressors, such as those of collagen type XVIII alpha 1 chain (COL18A1), collagen type 1 alpha 2 chain (COL1A2), as well as that for natriuretic peptide receptor 3 have been downregulated. The treatment with this

mycotoxin also upregulated the following CYP isoforms: CYP26B1, CYP3A4, CYP27B1 and downregulated CYP1A1, CYP1B1, CYP19A1, CYP36A1, CYP4B1 [74].

The same study from Pauletto et al. [74] revealed that all analyzed glutathione-S-transferase genes, except those for omega 1 and pi1 isoforms, have been downregulated. The gene sets for TNF-α signaling via NF-kB, oxidative phosphorylation, DNA repair, inflammatory response, KRAS signaling, p53 pathway, PI3K-Akt-mTOR signaling, apoptosis and hypoxia have been upregulated by AFB1 treatment of BFH12 cells. In the same conditions, other gene sets for epithelial–mesenchymal transition, bile acid metabolism, estrogen response and heme metabolism have been downregulated.

Recently, based on transcriptomic data and post translational analyses, it was postulated that Toll-Like Receptor (TLR2) activation is involved in AFB1-induced inflammation and oxidative stress in BFH12 cells [75]. Moreover, in a chicken hepatocarcinoma cell line (LMH) exposed to AFB1 differentially, expression analysis revealed that 1006 genes have been upregulated and 791 downregulated, compared with the control treatment. The mRNA expression of CYP27A1, CYP1A4, FABP2, PPARα and GSTT1 were significantly decreased by this mycotoxin treatment, whereas genes responsible for focal adhesion and MAPK pathways were upregulated compared with control ones [76].

Previously it was noticed that in HepG2 cells treated with AFB1, increases in the expressions of miR-34A and miR-33a-5p led to an important decrease of β-catenin, c-myc and cyclin D1 levels in the Wnt signaling pathway, generating an important risk of hepatocellular carcinoma [77][78]. The exposure to this mycotoxin also inhibits protein synthesis and due to this, enzymes` levels of different metabolic pathways are affected [79].

Recently, it was proved that AFB1 exposure of cells affects the respiratory chain, generating reactive oxygen species (ROS). If these are not counteracted by the antioxidant enzymatic and non-enzymatic systems, oxidative stress occurs [80]. The excess of ROS attacks polyunsaturated fatty acids from glycerophospholipids, generating end products of lipid peroxidation, as well as DNA and proteins. Lipid peroxidation and oxidative damage to DNA play a major role in the toxicity of aflatoxins.

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