

Review

Aflatoxicosis: Lessons from Toxicity and Responses to Aflatoxin B₁ in Poultry

Melissa S. Monson ¹, Roger A. Coulombe ² and Kent M. Reed ^{1,*}

¹ Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St Paul, MN 55108, USA; E-Mail: monso181@umn.edu

² Department of Animal, Dairy and Veterinary Sciences, College of Agriculture, Utah State University, Logan, UT 84322, USA; E-Mail: roger@usu.edu

* Author to whom correspondence should be addressed; E-Mail: reedx054@umn.edu; Tel.: +1-612-624-1287; Fax: +1-612-624-0204.

Academic Editor: Wayne L. Bryden

Received: 12 May 2015 / Accepted: 1 September 2015 / Published: 8 September 2015

Abstract: This review is a comprehensive introduction to the effects of poultry exposure to the toxic and carcinogenic mycotoxin aflatoxin B₁ (AFB₁). The relationship between AFB₁ sensitivity and metabolism, major direct and indirect effects of AFB₁, recent studies of gene expression and transcriptome responses to exposure, and mitigation strategies to reduce toxicity are discussed. Exposure to AFB₁ primarily occurs by consumption of contaminated corn, grain or other feed components. Low levels of residual AFB₁ in poultry feeds can cause reduction in growth, feed conversion, egg production, and compromised immune functions, resulting in significant economic costs to producers. Thus, AFB₁ acts as a “force multiplier” synergizing the adverse effects of microbial pathogens and other agents, and factors detrimental to poultry health. Domestic turkeys (*Meleagris gallopavo*) are one of the most sensitive animals known to AFB₁ due, in large part, to a combination of efficient hepatic bioactivation by cytochromes P450 1A5 and 3A37, and deficient hepatic glutathione-S-transferase (GST)-mediated detoxification. Because of their sensitivity, turkeys are a good model to investigate chemopreventive treatments and feed additives for their ability to reduce AFB₁ toxicity. Transcriptome analysis (RNA-seq) of turkey poult (liver and spleen) has identified AFB₁-induced gene expression changes in pathways of apoptosis, carcinogenesis, lipid regulation, antimicrobial activity, cytotoxicity and antigen presentation. Current research focuses on further identifying the molecular mechanisms

underlying AFB₁ toxicity with the goal of reducing aflatoxicosis and improving poultry health.

Keywords: turkey; aflatoxin B₁; hepatotoxicity; immunosuppression; feed additives; transcriptome; RNA-seq

1. Introduction

Dietary exposure to aflatoxins can have severe toxic and carcinogenic effects in humans and animals. The production and metabolism of aflatoxin, symptoms and biomarkers of exposure, and methods to reduce aflatoxicosis have been extensively investigated over the past 50 years [1–9]. Most studies have focused on humans, laboratory model species, or agricultural animals, and have identified conserved and species-specific aspects of aflatoxicosis [2,10–15]. This review specifically examines the responses of poultry, a particularly sensitive group, to aflatoxin B₁. Aflatoxin metabolism, toxicity, and expression responses in poultry are discussed, along with potential mitigation strategies.

2. Etiology of Aflatoxicosis

The acute toxicity of dietary aflatoxin was discovered in 1960 when a then unknown disease, termed Turkey “X” Disease, caused the deaths of over 100,000 turkeys (*Meleagris gallopavo*) and other poultry in England [16,17]. Upon examination, the causative agent was identified as imported Brazilian peanut-meal contaminated with aflatoxins [16,18]. Aflatoxins belong to a heterologous group of fungal secondary metabolites called mycotoxins that adversely affect human and animal health. Structurally derivatives of difurocoumarin, aflatoxins are most commonly produced by strains of *Aspergillus flavus*, *A. parasiticus*, and *A. nomius*, although many other *Aspergilli* (including *Emericella* teleomorphs) have aflatoxigenic capabilities [7,19–21]. Named according to their blue or green fluorescence under UV light, there are four primary aflatoxins: aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁), and G₂ (AFG₂) (Figure 1) [7,21]. Of these, AFB₁ is the most hepatotoxic, the most mutagenic, and the most prevalent worldwide [2,3,21,22].

Livestock, including poultry, are exposed to AFB₁ and other aflatoxins by consuming contaminated feed. Many agricultural feed commodities (corn, cottonseed, peanuts and sorghum) and other foods (figs, tree nuts and spices) are at especially high risk [21,23]. Stress from drought or insect damage can reduce crop resistance to *Aspergilli* and lead to aflatoxin contamination prior to harvest [21,24]. Warm and humid conditions during maturation, harvest, transport or storage, promote *Aspergillus* colonization and subsequent aflatoxin production [21,24]. Temperatures near 30 °C and water activity of 0.99 provide ideal conditions for AFB₁ biosynthesis, although substrate, time, CO₂ levels, and other environmental factors are also important [25–30]. Along with primary contamination of crops, aflatoxins can transfer to milk, meat and eggs of livestock and poultry fed the toxins [23,31–38]. Therefore, AFB₁ is a human food safety risk in both plant and animal products.

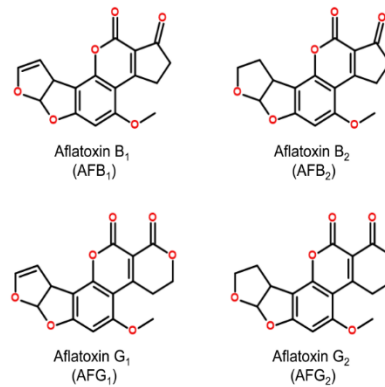


Figure 1. Molecular structures of the four primary aflatoxins.

Animal susceptibility to the acute effects of aflatoxicosis varies widely (Table 1). Domestic turkeys and ducks (*Anas platyrhynchos*) are highly sensitive to both the acute and chronic toxicity of AFB₁ [21,39,40]. Chickens (*Gallus gallus*) are more resistant to acute aflatoxicosis than other poultry species, except during embryonic development (Table 1). Even when exposure does not cause mortality or morbidity, aflatoxicosis contributes directly and indirectly to losses for the poultry industry. While precise numbers are not available, it has been estimated that aflatoxins inflict a loss of at least \$143 million each year due to AFB₁-induced hepatotoxicity, reduced performance and secondary infections [23,41].

Table 1. Comparative acute toxicity of a single oral dose of aflatoxin B₁ (AFB₁).

Species	Age	Oral LD ₅₀ (mg/kg Body weight) ¹
Baboon	A	2.0–2.2
Cat	A	0.6
Chicken	E	0.3–5.0
Chicken	Y	6.5–18.0
Dog	A	0.5–1.0
Duck	E	0.5–1.0
Duck	N	0.3–0.6
Guinea Pig	Y	1.4–2.0
Hamster	Y	10.2–12.8
Macaque (Cynomolgus)	A	2.2
Macaque (Rhesus)	A	7.8–8.0
Mouse	N	1.5
Mouse	Y	7.3–9.0
Rabbit	Y	0.3–0.5
Rat	N	0.6–1.0
Rat	Y	5.5–7.4
Rat	A	6.3–18.0
Sheep	A	2.0
Swine	Y	0.6
Trout	Y	0.5
Turkey	Y	1.4–3.2

Lethal dose in 50% (LD₅₀), adult (A), embryo (E), neonate (N), young (Y); ¹ compiled from [7,10–12,14,22].

3. AFB₁ Metabolism and Sensitivity

3.1. Metabolism

Bioactivation is required for AFB₁ to be toxic and this processing predominantly occurs in hepatocytes [1,4,7,21]. AFB₁ is initially absorbed in the small intestine, especially the duodenum [42]. While bioactivating enzymes with low affinity for AFB₁ are present in the small intestine [43], the majority of the toxin is metabolized in the liver, where AFB₁ is converted by hepatic cytochromes P450 (CYP) enzymes into the reactive and electrophilic *exo*-AFB₁-8,9-epoxide (AFBO) (Figure 2) [1,3,4,21,22,40]. An *endo* stereoisomer of the AFBO epoxide can also be produced, but is far less toxic and not relevant to AFB₁ toxicity [1,4].

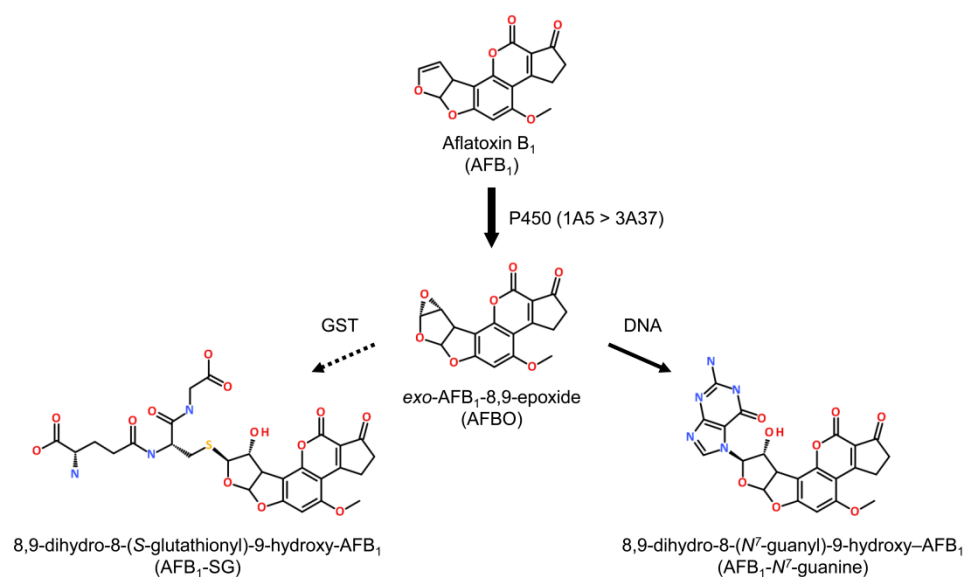


Figure 2. Metabolites and enzymes involved in aflatoxin B₁ (AFB₁) metabolism in the turkey liver. Arrow width shows reaction efficiency (wide > narrow), while a dashed line indicates that a reaction does not occur in all birds. Cytochrome P450 (CYP) enzymes effectively interact with AFB₁. Domestic turkey glutathione *S*-transferase (GST) enzymes cannot conjugate *exo*-AFB₁-8,9-epoxide (AFBO), although wild and heritage turkey GST enzymes can have activity.

AFBO is a highly unstable intermediate ($t_{0.5} \sim 0.5$ s) and quickly reacts to form adducts with DNA, RNA, and proteins [1,4,7], which are then responsible for AFB₁ toxicity. In most mammals, AFBO is primarily detoxified by glutathione *S*-transferase (GST) enzymes that add glutathione (GSH) to form an 8,9-dihydro-8-(*S*-glutathionyl)-9-hydroxy-AFB₁ (AFB₁-SG) adduct (Figure 2) [3,4,7,21,40]. Although AFBO is the most toxic, other metabolites of AFB₁ include aflatoxicol (AFL), aflatoxin M₁ (AFM₁), aflatoxin P₁ (AFP₁), and aflatoxin Q₁ (AFQ₁) [3,4,7].

3.2. Sensitivity: Mice to Humans

Sensitivity to AFB₁ is determined not only by the rate of P450-mediated bioactivation, but more importantly by the subsequent detoxification of the reactive AFBO intermediate [1,4]. In mice

(*Mus musculus*), P450 enzymes such as P450 2A5, 3A11 and 3A13, effectively activate AFB₁ and can produce large amounts of AFBO [4,44–50]. However, the murine alpha-class GST (GSTA) enzyme mGSTA3 has high affinity for AFBO, making mice extremely resistant to aflatoxicosis [4,45,51–55]. Interestingly, the orthologous hepatic GST enzymes from rats (*Rattus norvegicus*) can have more than 50 times lower activity towards AFBO [45,54,56]. Effective activation by rat P450 3A2 and 2C11 and minimal conjugation by the rat orthologue of mGSTA3 (rGSTA3, Yc1) make the rat far more susceptible to aflatoxicosis [4,44,45,51,55,57–59]. Another rat AFBO-conjugating enzyme rGSTA5 (Yc2) has high affinity for AFBO, but expression is limited to neonatal tissue, some female adults and after antioxidant-induction [59–62]. Therefore even within rodents, the metabolism and sensitivity to AFB₁ is species-specific.

In humans (*Homo sapiens*), multiple hepatic P450 enzymes can activate AFB₁, but P450 1A2 and 3A4 are the primary producers of AFBO [4,43,63–67]. Human P450 3A4 is active at high AFB₁ concentrations, while P450 1A2 has high affinity at low biologically-relevant concentrations [64,66]. Overall, human P450 enzymes produce far less AFBO than rodents, even at high concentrations of AFB₁ (1/4 that of rats, 1/8 that of mice) [48]. Although lower levels of AFBO reduce toxicity, the epoxide is less effectively detoxified in humans. Hepatic GST enzymes in humans can have over 3000 fold less activity against AFBO than murine GST [56]. Human enzymes hGSTA1 and hGSTA2 have little AFBO-conjugating activity [57,59,68]. Instead, some AFB₁-SG adducts are produced by the mu-class GST (GSTM) enzyme hGSTM1 [59,68–71]. Although hGSTM1 forms more AFB₁-SG adducts from the *endo* stereoisomer, it is able to detoxify mutagenic *exo*-AFBO [68].

3.3. Sensitivity: Poultry

Poultry are sensitive to even low levels of AFB₁, and among species of agricultural importance, the order of sensitivity is ducks > turkeys > Japanese quail (*Coturnix japonica*) > chickens [7,21,72–75]. Therefore, lower concentrations of AFB₁ are lethal to turkeys and ducks and more adversely affect production and health in these species (Table 2). In domestic turkey, efficient production of AFBO contributes to sensitivity. We have conducted considerable research to determine the P450 enzymes responsible for AFB₁ bioactivation and metabolism in turkey livers. This work revealed two turkey P450 enzymes, encoded by *CYP1A5* and *CYP3A37*, predominantly responsible for converting AFB₁ into AFBO *in vitro* and *in vivo* [76–79]. P450 1A5 has high-affinity (high V_{max}, K_{cat}; low K_m) and catalyzes the production of both *exo*-AFBO and the detoxified metabolite AFM₁ according to traditional Michaelis-Menten kinetics [76,77]. P450 3A37 is the lower affinity catalyst, exhibiting apparent subunit allostery conforming to Hill enzyme kinetics and producing *exo*-AFBO and AFQ₁ [76,77].

We used polyclonal anti-peptide antibodies in a series of immunoinhibition experiments to determine the relative importance of these P450 enzymes in AFB₁ bioactivation in turkey livers and liver microsomes. Turkey P450 1A5 is the predominant catalyst (>98%) at low (<50 μM) pharmacologically-relevant AFB₁ concentrations commonly seen in the livers of exposed turkeys, while P450 3A37 predominates at much higher AFB₁ concentrations not likely to be achieved in tissues under *in vivo* conditions [78]. Another P450 enzyme, likely orthologous to mammalian P450 2A6, also converts AFB₁ to AFBO in turkeys, chickens, ducks and quail [80–82]. However, the specific gene encoding this protein has not been identified in any poultry species [80–83]. These same studies

implicated a P450 1A1 orthologue in AFBO production, which is likely encoded by *CYP1A5* in turkeys [78,80–82].

Table 2. Minimum AFB₁ concentrations with major effects in poultry.

Species	Minimum Dietary Contamination Level (ppb) to Cause		
	100% Lethality	Gross Hepatic Lesions	Impaired Production
Chicken	NR (>4000)	800 ¹	800 ¹
Duck	1000 ²	500 ²	500 ²
Goose	4000 ²	500 ²	700 ³
Pheasant	4000 ²	500 ²	1000 ²
Quail (Bobwhite)	ND	ND	700 ³
Turkey	800 ¹	400 ¹	400 ¹

Compiled from studies that examined aflatoxicosis in multiple poultry species; parts per billion (ppb); not determined (ND); not reached (NR); ¹ [39]; ² [75]; ³ [72].

The activity of P450 enzymes in the turkey, and therefore AFBO production, is inversely related to age, with extreme sensitivity to AFB₁ in young poultts [39,77,84,85]. A similar age-dependent sensitivity occurs in chickens [86–88]. Hepatic microsomal P450 enzymes from turkeys and quail produce 2 to 4 times more AFBO than ducks or chickens [74]. A later study confirmed the highest AFBO production in turkey, with intermediate levels in duck and quail, and the lowest in chicken [83]. AFBO production correlates with the sensitivity of poultry, except for the duck. In addition, the levels of AFL produced by liver cytosol are highest in turkey, followed by ducks, chickens and quail [74]. The sensitivity of ducks may be more linked to AFL production and toxicity or to oxidative stress [74,83,89].

Like the cytochrome P450 enzymes, hepatic tGSTA enzymes contribute to the severity of aflatoxicosis in the turkey. Six *tGSTA* genes have been amplified, cloned and heterologously expressed turkey liver (*GSTA1.1*, *GSTA1.2*, *GSTA1.3*, *GSTA2*, *GSTA3*, and *GSTA4*) [90,91]. Hepatic cytosolic GST enzymes from domestic turkey have essentially no AFBO-conjugating activity [40,91]. However, tGSTA enzymes can conjugate AFBO to GSH *in vitro* using recombinant proteins in an *E. coli* expression system, suggesting that gene silencing mechanisms or post-transcriptional modifications are likely responsible for their lack of function *in vivo* [91]. The high sensitivity of the domestic turkey to AFB₁ appears to be due to an unfortunate combination of efficient P450 enzymes and dysfunctional GST enzymes that allow accumulation of AFB₁ adducts in the liver.

The effects of AFB₁ exposure on North American wild turkeys are similar to, but less severe than those seen in domestic poultry [92]. This difference in response may be the result of genetic changes that occurred during domestic selection, but could also be due to the separate genetic background of the domestic turkey. Although members of the same species, domestic turkeys were originally derived from the Mexican subspecies (*Meleagris gallopavo gallopavo*) of wild turkey native to Central America; the Eastern subspecies from North America (*Meleagris gallopavo silvestris*) was likely involved in later crosses [93]. Wild turkeys were originally exported to Europe from Mexico in the 1500's and then reintroduced to North America in the 1600's. Reintroduced turkeys were selectively bred, first forming the heritage breeds, and then developed into the modern commercial breeds. Current commercial production predominately utilizes the Broad Breasted White. Selection for production traits in poultry is

known to increase metabolic disorders [94] and decrease immune functions [95–98]. Likewise, detoxification capabilities could be reduced as an unintended side-effect of selection in domestic birds.

Eastern wild turkeys are more resistant to aflatoxicosis than their domestic relatives [92]. AFBO detoxification is likely a main contributor to the differences seen among turkeys. Consistent with this hypothesis, hepatic cytosolic GST enzymes from both the Eastern and Rio Grande (*Meleagris gallopavo intermedia*) subspecies of wild turkey have activity against AFBO [99], unlike their domestic counterparts. Wild turkey GSTA enzymes heterologously expressed *in vitro* also had AFBO-conjugating activity [99]. Like wild strains, the Royal Palm turkey, a heritage breed, retains hepatic and *in vitro* activity [99]. tGSTM enzymes were also amplified, cloned and heterologously expressed from the livers of wild and domestic turkeys, but had no measurable affinity toward AFBO, and therefore are not thought to be involved in AFB₁ detoxification [100].

4. Effects of AFB₁ Exposure

4.1. AFB₁ Adducts

Formation of AFB₁ adducts is detrimental to cellular processes. AFBO can react with DNA or RNA to form *trans*-8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB₁ (AFB₁-N7-guanine) adducts (Figure 2) [1,3,4,7,21]. Some AFB₁-N7-guanine adducts convert into stable AFB₁-formamidopyrimidine (AFB₁-FAPY) adducts [1,3,7,101]. These AFB₁-DNA and AFB₁-RNA adducts can inhibit transcription and translation, induce DNA mutations during DNA repair and replication, and even initiate apoptosis or carcinogenesis [1,3,4,21,44,102]. AFBO can hydrolyze into AFB₁-8,9-dihydrodiol (AFB₁-diol) and through a series of reactions generate adducts with lysine residues in proteins [4,89,103]. AFB₁-lysine adducts can cause toxicity by impairing protein stability and function [4,89,103]. Although not as well studied, AFB₁ adducts have similar effects on poultry. In chicken primary hepatocytes, the interaction between AFBO and DNA, RNA and proteins has been verified and shown to strongly inhibit synthesis of these macromolecules [104].

4.2. Mutagenicity

Binding of AFBO to DNA introduces G-T transversion mutations in hepatic DNA [4,65,105]. The high incidence of G-T transversions results from AFB₁-FAPY adducts predisposing bypass DNA synthesis machinery to make G-T changes [101]. A G-T transversion in codon 249 of the p53 tumor suppressor has been identified in many human liver cancers and may mechanistically contribute to cancer formation [7,65,71,101,105–107]. Chronic AFB₁ exposure, especially in combination with hepatitis-B infections, severely increases the risk of hepatocellular carcinoma in humans [1,3–6,9,21,105,108]. Dietary AFB₁ is known to have hepatocarcinogenic effects in other mammals, especially the sensitive rat [4,109–111]. Based on this evidence from humans and other mammals, AFB₁ is classified as a group I carcinogen by the International Agency for Research on Cancer [4,105]. AFBO is highly mutagenic in poultry, although adenoma and hepatocellular carcinoma have only been reported in ducks [82,112–114]. The potential synergistic effect of hepatitis-B virus and AFB₁ has not been consistently reproduced in these studies [112–114].

4.3. Production Losses

Beyond its mutagenic effects, AFB₁ negatively affects production values, resulting in economic losses for the poultry industry. Dietary exposure to AFB₁ and other aflatoxins leads to lower weight gain and absolute body weights in both chickens and turkeys [34,39,84,88,115–124]. Reduced feed intake [34,115,118,122–125] and decreased efficiency of nutrient usage [34,39,84,88,115,122,125] both contribute to this impaired growth during aflatoxicosis. AFB₁ lowers feed conversion causing poultry to require more feed to produce muscle (broilers and turkeys) [39,84,88,115,122] and eggs (layers) [125]. Although less severe, AFB₁ also reduces feed intake and weight gain in wild turkeys [92]. Similarly, feed consumption was decreased in AFB₁-exposed quail, but body weight and feed conversion were unaffected [126]. In ducks fed AFB₁, both feed intake and weight gain were reduced but without affecting feed efficiency [127].

Exposure to aflatoxins lowers reproductive performance in poultry. In layers fed AFB₁, age to maturity is increased [34] and egg production is reduced [34,121,125,128–131]. Egg quality parameters such as total weight, shape, albumin or yolk percentage, and shell thickness in chickens and quail can be adversely affected by AFB₁, although the effects were variable among studies [34,125,126,128,131–133]. The declines in poultry production traits are often indirect effects of AFB₁ reducing the metabolic potential of the liver. For example, impaired hepatic protein production likely contributes to AFB₁-induced changes within eggs, as the liver is the chief site of synthesis of proteins and lipids incorporated into the egg yolk.

4.4. Hepatotoxicity

Critical to protein synthesis, enzymatic metabolism and detoxification processes, the liver is the primary site of AFB₁ activation and therefore toxicity [1,4,7,21]. Aflatoxicosis in poultry is characterized by an enlarged, pale, and friable liver [10,34,38,39,84,116,117,120,121,134,135]. Although relative liver weight can initially decrease [117], longer exposure to dietary AFB₁ raises the relative weight of the liver and causes pale or yellowed pigmentation [10,84,88,115–117,120–124,135,136]. At the cellular level, increased vacuolation of AFB₁-exposed hepatocytes allows high levels of lipids to accumulate [10,34,39,84,116,120,121,126]. Steatosis is therefore responsible for the changes in liver color and size during aflatoxicosis.

Both acute and chronic AFB₁ consumption by poultry cause other hepatic lesions. Common histopathological signs of AFB₁-induced liver damage include focal necrotic hepatocytes or hemorrhages [10,34,39,84,116,121,137]. Acute damage initiates inflammatory responses and leads to leukocyte infiltration and proliferation in the liver [10,34,84,112,116,138]. Short-term exposure to higher dose can cause morbidity and mortality from extensive liver damage [3,21]. In poultry, chronic AFB₁ consumption is mutagenic and leads to remodeling of liver tissues. Hyperplasia of bile duct epithelial cells or oval cells develops first, followed by periportal fibrosis and nodular tissue regeneration [10,34,39,84,112,116,121,126,134,139].

AFB₁ adducts with biomolecules cause damage to hepatocytes that impairs metabolic functions of the liver during AFB₁ exposure. This is exemplified by AFB₁-reduced total serum protein levels, as the liver is responsible for production of most circulating proteins [88,115,117–119,123,127,129,140,141]. Aflatoxicosis negatively affects albumin, globulin, cholesterol, and triglyceride levels in

serum [88,115,117,119,127,129,136,140–142]. Multiple blood coagulation factors are produced in the liver; the activity of these clotting factors and the serum levels of fibrinogen are diminished by AFB₁ in both chickens and turkeys [142–146]. Hepatic protein concentrations also decrease in AFB₁-fed chickens [88]. Protein content likely declines because AFB₁-DNA adducts inhibit transcription or translation and AFB₁-lysine adducts result in protein degradation or excretion. Reduced synthesis of enzymes in the liver would have systemic effects on poultry metabolism. For example, the decreased hepatic fatty acid synthesis observed in AFB₁-exposed chickens [147] could be responsible for lower production of serum cholesterol and triglycerides [129,140].

4.5. Immunotoxicity

The avian immune system relies on the bursa of Fabricius, thymus and spleen to produce mature or active leukocytes. Even at low dietary concentrations, AFB₁ can damage these immune tissues and suppress innate and adaptive immune responses [3,7,148]. AFB₁ consumption during growth can lead to immune tissue atrophy, reducing relative weights of the bursa, spleen and thymus [120,122,134,139,149–152]. Increases in relative spleen weight have also been observed during aflatoxicosis [117,120,153–156]. In young chickens, tissue changes are concomitant to the development of histopathological lesions. AFB₁ exposure causes visible congestion in the spleen and thymus, while nuclear debris accumulates in the thymus and bursa [139,149,150,156]. In AFB₁-exposed chicks, vacuoles increase in the lymphoid follicles of the bursa and the white pulp of the spleen, especially the T-cell rich periarteriolar lymphoid sheaths [139,150,156].

At the cellular level, innate and adaptive cell-mediated immune functions are impaired by AFB₁ exposure in poultry [148]. *In vivo* exposure to AFB₁ and other aflatoxins has been shown to decrease phagocytic activity in chicken leukocytes, including heterophils [157,158], macrophages [159–161], and monocytes [162]. Reduced phagocytosis was demonstrated *in vitro* for peritoneal macrophages isolated from both chickens and turkeys, although microsomally activated AFB₁ was required [163,164]. In contrast, the phagocytic activity of thrombocytes was not affected in chickens fed an aflatoxin-contaminated diet [165]. Dietary AFB₁ inhibits T lymphocyte activation in both chickens and turkeys as evidenced by delayed hypersensitive skin tests and graft-versus-host response tests [39,84,116,122,159,166,167].

AFB₁ can induce circulating lymphocytopenia [34,159,166,168] and cause lymphoid depletion in the bursa, spleen and thymus [34,134,139,149,150,159,166]. This likely results from increased apoptosis of splenocytes, thymocytes and bursal B-cells as seen in young chickens during aflatoxicosis [139,149,156,169,170]. In the chicken, both CD4⁺ and CD8⁺ T lymphocytes in the spleen, thymus, peripheral blood, and even the ileum can be affected by AFB₁ exposure [149,150,156,168,171,172]. Oxidative stress and DNA damage from AFB₁ are likely responsible for initiating apoptotic processes in lymphocytes [169,170]. Together these losses reduce the adaptive immune potential of poultry fed AFB₁.

Although some studies only observed effects on cell-mediated immunity [39,84,116], others found that AFB₁ can similarly diminish innate and adaptive humoral immune capabilities. Total serum complement is decreased by feeding AFB₁ to chickens, ducks and turkeys [115,127,173–175]. Antibody titers are often reduced, whether measured as total serum levels of IgA, IgG and IgM [139,141,167], as

production of specific antibodies in response to sheep red blood cells [122,152,159] or as exposure response to infectious bronchitis virus, infectious bursal disease virus, Newcastle disease virus or *Pasteurella multocida* [128,176,177]. AFB₁ can also impair the effectiveness of vaccination for these poultry diseases [128,158,176–178].

As a consequence of AFB₁-driven immunosuppression, exposed poultry have lower resistance to secondary infections [148]. Poultry with aflatoxicosis are more susceptible to the bacterial diseases, fowl cholera [151,176,179–181] and salmonellosis [182–184]. Exposure to AFB₁ can increase the severity of the protozoan disease, cecal coccidiosis [185–188] and the fungal infection, crop mycosis [189]. Similarly, AFB₁ consumption decreases resistance to viral pathogens, including infectious bronchitis virus [128,177], infectious bursal disease virus [128,176,177,190,191], Marek's disease virus [185,192], and Newcastle disease virus [128,158,177,178,193]. These secondary infections dramatically increase the economic losses attributed to AFB₁ exposure. Thus, AFB₁ is a potent immunotoxin and acts as a synergistic “force multiplier” that can enhance the incidence and impacts of avian diseases.

4.6. Intestinal Toxicity

Nutrients are primarily absorbed by epithelial cells in the small intestine and these cells facilitate uptake of AFB₁. Most AFB₁ is transferred directly into the blood stream; however, as in mammals [43], some may be metabolized in intestinal tissues. Therefore in poultry, both local and systemic effects of AFB₁ exposure likely occur in the small intestine, but these are not well characterized. Dietary exposure to AFB₁ can lower the unit weight (length/weight) of the duodenum and jejunum [194,195] and affect tissue morphology. In chickens, AFB₁ has been shown to raise crypt depth in the jejunum [196], decrease villus height in the duodenum [197], and lower the ratio between villus height/crypt depth in all three sections of the small intestine [197]. However, these histopathological effects may not be pervasive [198].

The severity of aflatoxicosis in poultry can be affected by nutrition [7]. Deficiencies in some dietary vitamins raised AFB₁ sensitivity in chickens, although feeding vitamins in excess was not protective [199,200]. Dietary supplementation with tryptophan also increased hepatotoxicity of AFB₁ [201]. Conversely, diets high in fat or protein may be beneficial to chickens and turkeys fed AFB₁ [202–204].

Direct investigations of AFB₁-effects on absorption or retention of individual nutrients have had variable results [34,194,196,205–208]. Exposure to AFB₁ decreases the apparent metabolizable energy poultry can obtain from their diet [34,195,196,205–207]. Therefore, increased dietary nutrients are needed to compensate for impaired uptake. It is currently unclear how much reduced nutrient uptake in the intestine contributes to AFB₁ effects on growth and feed efficiency in poultry.

4.7. Embryotoxicity

Exposure during development recapitulates many of signs of aflatoxicosis seen in hatched chicks and poults. Although most studies are carried out by *in ovo* AFB₁ injections, embryonic exposure to the toxin is a known risk to poultry. AFB₁ and its metabolites can be transferred from the laying hen into the albumin and yolk of the egg [31,34,37,128,131,133,209]. AFM₁ is a common metabolite detected in eggs and, while not as carcinogenic as AFB₁, is acutely toxic [4,37,133]. Contamination of unfertilized shell eggs is therefore a food safety risk when used for human consumption.

Transfer of aflatoxins into embryonated eggs is also a concern for poultry producers. In experimental settings, *in ovo* exposure of chickens or turkeys to AFB₁ caused DNA damage in the embryonic liver and increased embryo mortality [131,161,210–215]. When introduced into the maternal diet to simulate the natural route of embryonic exposure, AFB₁ caused reduced hatchability [130,131,161]. Consistent with studies in hatched poultry, turkeys may be more sensitive to embryotoxic effects than chickens [215]. Since embryonic liver has an active protein from the *CYP1A* family [216], hepatotoxicity in turkey embryos is likely mediated by the same P450 1A5 as in poult.

Embryonic AFB₁ exposure can lead to morphological defects [212], such as abnormal area opaca cells [210,214], skeletal defects in the tibia growth plate [213], and inhibition of bursal follicle development [210,214]. These mutagenic effects can reduce embryo viability and adversely affect hatched progeny. *In ovo* AFB₁-driven immunosuppression has the potential to increase the incidence of infectious disease in young poultry and negatively affect their health and productivity. Whether *in ovo* injection or maternal feeding, chickens exposed to AFB₁ during embryogenesis have compromised cellular and humoral immune functions post hatch [160,161,211,217,218].

5. Gene Expression and AFB₁

5.1. P450 and GST Enzymes

Gene expression can improve our understanding of responses to AFB₁ and provide targets to modulate the mechanisms of toxicity. Both cellular responses to toxicity and AFB₁ inhibition of transcription and translation will affect gene expression [102]. For example, AFB₁ down-regulates *p53* expression in human cells [67] and in tissue from hepatocellular carcinomas in rats [219], likely due to mutations introduced by AFB₁-DNA adducts. Expression of AFB₁-metabolizing genes can also be affected by AFB₁. Exposure in the rat liver to AFB₁ can increase expression of P450 (*CYP3A*, *CYP4F1*), and *rGSTM2* genes (Yb2) [220]. Another study in rats identified effects on multiple *P450* and *GST* genes, with greatest up-regulation in *rGSTA5* and pi-class GST (GSTP) *rGSTP1* [221]. In chickens, dietary AFB₁ up-regulated hepatic expression of *CYP1A1* and *CYP2H1*, and down-regulated expression of epoxide hydrolase (*EH*) and *GSTA*, although the specific *GSTA* gene target was not identified [123,124]. Other *P450* family members were significantly down-regulated [124]. Interestingly, a recent study in broilers observed the opposite changes in the liver, down-regulation of *CYP1A1* and up-regulation of *EH* and *GST* [115].

5.2. Cytokines and the MHC

AFB₁ is known to initiate hepatic inflammation, and correspondingly, was shown to affect expression of pro-inflammatory cytokines in both mammals and poultry [124,171,222–226]. Splenic expression of interleukins 1 beta (*IL1β*), 6 (*IL6*), 10 (*IL10*), interferon gamma (*IFN-γ*) and tumor necrosis factor alpha (*TNF-α*) in pigs (*Sus scrofa*) were increased by AFB₁ exposure [225]. Expression of *IFN-γ* and *TNF-α* increased in rats during aflatoxicosis, and protein levels of IL-1, IL-2, and IL-6 were also modulated [224,226]. In chickens, dietary AFB₁ increased hepatic expression of *IL6* [115,124]. However, in another experiment [123], expression of the IL6 receptor (*IL6R*) and IL10 receptor beta (*IL10RB*) were reduced in the liver. Exposure to AFB₁ reduced ileac expression of *IL2*, interleukin 4 (*IL4*), *IL6*,

IL10, interleukin 17 (*IL17*), and *IFN-γ* in chickens [171,172]. Lipopolysaccharide-induced TNF factor (*LITAF*) expression also decreased in the intestine [171,172]. This gene is used as a marker for TNF activity since *TNF-α* has not been identified and may not be present in birds. Lastly, serum protein levels of IL-2 and *IFN-γ* were lower in chickens exposed to AFB₁ [168]. Down-regulation of these cytokines and their receptors in poultry may result in decreased T lymphocyte activation and proliferation.

Both up- and down-regulation of major histocompatibility complex (MHC) class I genes in response to AFB₁ exposure was observed in pigs [227], rats [221], and rainbow trout (*Oncorhynchus mykiss*) [228]. The MHC is a highly polymorphic genomic region that contains genes encoding proteins essential to innate and adaptive immune functions. For example, MHC class I and class II molecules are necessary for antigen presentation to T lymphocytes and are common to the galliform MHC [229–237]. In turkeys and chickens, the MHC is composed of 2 genetically unlinked regions, the *B*-locus (*MHC-B*) and *Y*-locus (*MHC-Y*), co-located on a single microchromosome (GGA16 or MGA18, respectively) [229–237]. Although some genes are well characterized, the functions and expression patterns of many poultry MHC genes are still unknown. In the turkey, expression of multiple MHC genes significantly increased in response to AFB₁ exposure [238].

5.3. Moving towards Transcriptomics

Few studies have examined gene expression changes across the entire transcriptome. Although the specific genes affected by AFB₁ vary between species, exposure to the toxin has an up-regulatory effect on expression of damage responses, or when those fail, pathways of carcinogenesis. In rats, AFB₁ enhanced hepatic expression of genes involved in xenobiotic detoxification, cell cycle regulation, oxidative stress, DNA damage repair, tumor development and amino acid metabolism [221]. For example, E2F transcription factor 1 (*E2F1*) and many genes downstream of *E2F1* were up-regulated by AFB₁ exposure; *E2F1* regulates DNA replication and apoptosis, and could contribute to carcinogenesis [221]. Aflatoxicosis in pigs affects expression of many of the same pathways, with greatest effects on metabolism, cell cycle, DNA damage responses and apoptotic processes in one study [239] and on protein degradation, metabolism, apoptosis, and immune responses in another [227]. Hepatocellular carcinomas from AFB₁-exposed rainbow trout showed changes in cell cycle, metabolism, immune and acute phase response genes when compared to adjacent non-cancerous liver tissue [228]. Two of these studies utilized high throughput RNA sequencing (RNA-seq), rather than microarrays, to characterize transcriptome changes after AFB₁ exposure [221,239].

5.4. Poultry Transcriptomics and AFB₁

AFB₁ effects on the transcriptome have been investigated in chickens using microarray [123]. Exposure to AFB₁ affected hepatic expression of genes associated with fatty acid metabolism, development, detoxification, coagulation, immunity and cell proliferation [123]. Among these, the greatest proportions of differentially expressed genes were involved in cell proliferation and metabolism. For example, aflatoxicosis had the greatest effect on expression of the cell signaling inhibitor Dickkopf homolog 3 (*DKK3*) (down-regulated) and the metabolic glycogen synthase 1 (*GYS1*) (up-regulated).

More recently, RNA-seq has been applied to the domestic turkey transcriptome to elucidate hepatic and splenic responses to dietary AFB₁ [227,238,240]. Similar to expression changes in other species,

transcriptome analysis in the turkey found that more genes were up-regulated than down-regulated by AFB₁ [227,238,240]. In one study of the liver, more than 80 genes belonging to cancer or focal adhesion pathways were up-regulated during aflatoxicosis, whereas smaller numbers of genes involved in cell signaling, cytoskeleton, and cell cycle were also up-regulated [227]. Conversely, the greatest numbers of down-regulated genes were involved in complement and coagulation.

Our investigation of AFB₁-hepatotoxicity in the turkey by RNA-seq found 313 transcripts significantly affected by AFB₁ exposure, with up-regulation of genes involved in apoptosis, cancer, and cell cycle regulation, and down-regulation of lipid metabolism [240]. Greatest up-regulation was observed for keratin 20 (*KRT20*), cell-death activator CIDE-3 (*CIDEC*), and E3 ubiquitin-protein ligase Mdm2 (*MDM2*). Alpha-2-macroglobulin (*A2M*) was the most down-regulated gene. Exposure to AFB₁ also turned on expression of HEPACAM family member 2 (*HEPACAM2*), a protein important in modulating cell adhesion and migration, and *S*-adenosylmethionine synthase isoform type-2 (*MAT2A*), important in methylation pathways.

Utilizing spleen samples from the same AFB₁-challenge trial, immunotoxicity in domestic turkey was also examined through RNA-seq [238]. Exposure to AFB₁ induced significant expression changes in 391 *de novo* assembled transcripts; the greatest up-regulation was seen in E3 ubiquitin-protein ligase CBL-B (*CBLB*) and ubiquitin specific peptidase 40 (*USP40*). Of the significantly altered transcripts, 27.6% encoded proteins with known immune functions representing both innate and adaptive responses. Antimicrobial genes, including beta-defensin 1 (*THP1*) and 2 (*THP2*), were down-regulated, while cytotoxic and antigen presentation genes, such as granzyme A (*GZMA*), perforin 1 (*PRF1*), MHC class IA and class IIB, were up-regulated in AFB₁-exposed tissue.

These studies were also designed to evaluate the ability of a *Lactobacillus*-based oral probiotic to reduce AFB₁-effects on the liver and spleen [238,240]. In the same challenge trial, domestic turkeys were exposed to probiotics alone or in combination with AFB₁. Addition of probiotics during AFB₁ exposure mitigated AFB₁-induced expression changes in genes such as serine/arginine repetitive matrix protein 1 (*SRRM1*), 28S ribosomal RNA (*RNA28S*), and ISG12-2 protein-like (*ISG12-2*) (Figure 3A) [240]. In the spleen, probiotics had greater ameliorating properties, significantly reducing AFB₁-effects on multiple immune genes, including *THP2*, *GZMA*, and *PRF1* (Figure 3B) [238]. However, probiotics were unable to reverse most AFB₁-induced expression changes and even had synergistic effects with AFB₁. For example, combined treatment increased differential expression in apolipoprotein A-IV (*APOA4*) in the liver and interestingly both *RNA28S* and *ISG12-2* in the spleen (Figure 3). Therefore, oral probiotics modulated expression in both tissues, but did not restore normal transcriptome profiles.

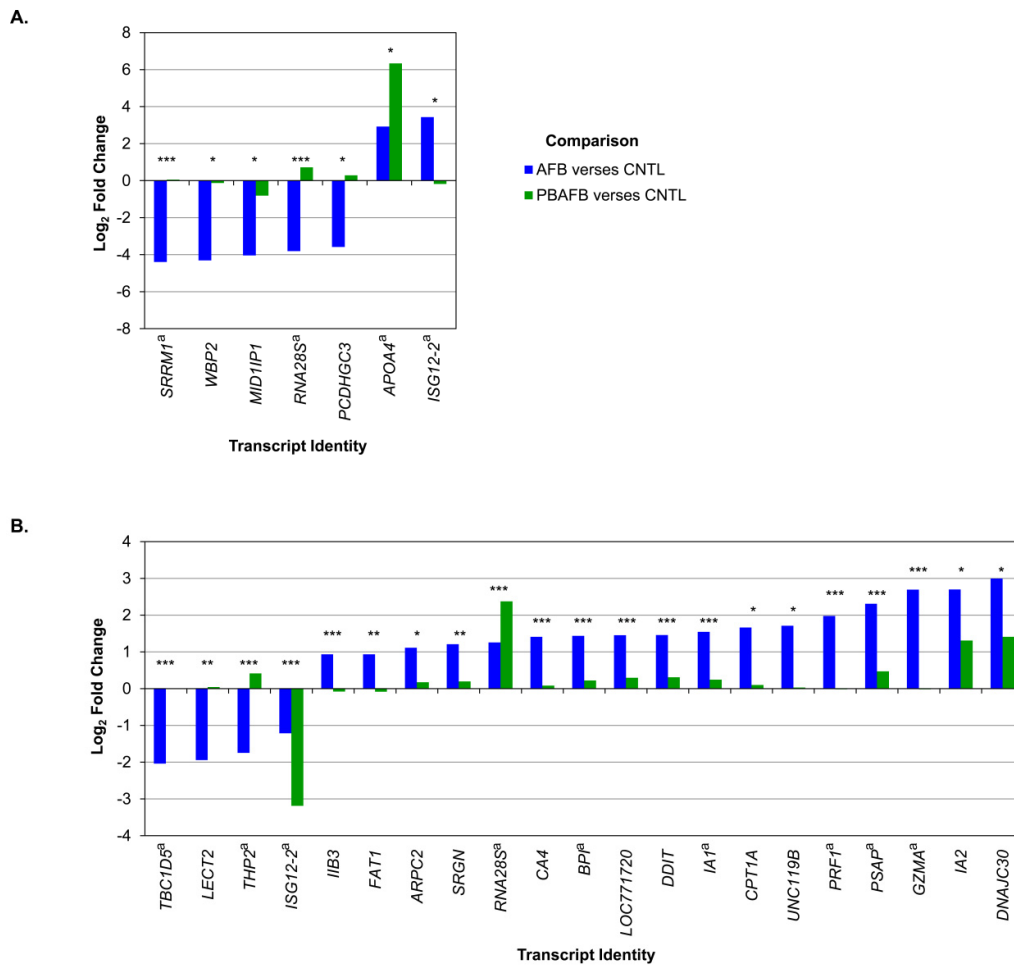


Figure 3. Oral probiotics mitigate gene expression changes induced by aflatoxin B₁ (AFB₁). (A) Liver. (B) Spleen. Graphs show annotated transcripts from each tissue with significant differential expression in the AFB₁-treated group (AFB) versus the control group (CNTL) and in the probiotic + AFB₁ group (PBAFB) versus AFB. Bars illustrate log₂ fold change in AFB versus CNTL (blue) or PBAFB versus CNTL (green). Significance of the probiotics (PBAFB versus AFB) is represented by the number of asterisks (*: 0.05 > p-value > 0.01, **: 0.01 > p-value > 0.001, ***: p-value < 0.001). Genes with multiple significant transcripts are indicated by ^a; only the most significant transcript in PBAFB versus AFB is shown. Apolipoprotein A-IV (*APOA4*), actin-related protein 2/3 complex subunit 2-like (*ARPC2*), bactericidal permeability-increasing protein (*BPI*), carbonic anhydrase IV (*CA4*), carnitine O-palmitoyltransferase 1, liver isoform (*CPT1A*), DNA-damage-inducible transcript 4 (*DDIT*), DnaJ homolog subfamily C member 30 (*DNAJC30*), FAT tumor suppressor homolog 1 (*FAT1*), granzyme A (*GZMA*), MHC class I antigen alpha chain 1 (*IA1*), MHC class I antigen alpha chain 2 (*IA2*), MHC class II antigen beta chain 3 (*IIB3*), ISG12-2 protein-like (*ISG12-2*), leukocyte cell-derived chemotaxin-2 (*LECT2*), uncharacterized LOC771720 (*LOC771720*), MID1 interacting protein 1 (*MIDIIP1*), protocadherin gamma subfamily C3 (*PCDHGC3*), perforin 1 (*PRF1*), presaposin (*PSAP*), 28S ribosomal RNA gene (*RNA28S*), serglycin (*SRGN*), serine/arginine repetitive matrix protein 1 (*SRRM1*), TBC1 domain family member 5 (*TBC1D5*), beta-defensin 2 (*THP2*), unc-119 homolog B (*UNC119B*), and WW domain-binding protein 2 (*WBP2*).

6. Strategies to Reduce AFB₁ Toxicity

6.1. Chemical Detoxification

Since prevention of AFB₁ contamination is often impractical, methods to chemically detoxify this mycotoxin have been intensely investigated [7]. Several candidate chemicals have been examined for their ability to detoxify AFB₁ in crops such as grain, rice, corn, and cottonseed. These include ammonium hydroxide [241–246], calcium hydroxide [243,247], hydrogen peroxide [243,248], sodium hydroxide [243,249], and sodium hypochlorite [243,250] all of which reduce AFB₁ concentrations through hydrolysis and produce a degraded form with reduced or no toxicity. However, most of these chemicals are of themselves, hazardous and safe use is often expensive and may decrease the nutrient value of feed components. In addition, it is not possible to fully preclude low levels of AFB₁ in feed production, especially in crops that are heavily contaminated, severely reducing returns for producers.

6.2. Feed Additives

Given that eliminating all potential for exposure to AFB₁ is not feasible, feed additives have been examined for their ability to protect poultry from aflatoxicosis [7,21]. Some additives, such as selenium supplementation, attempt to boost detoxification, metabolic, or immune functions to counteract the effects of AFB₁ [139,149,150,168,170,171,183,251]. However, most additives have been investigated for their potential to reduce AFB₁ uptake by the intestine [21]. Many natural absorbents have been shown to decrease the effects of AFB₁ in poultry, including super-activated charcoal [153], zeolites like hydrated sodium calcium aluminosilicate [115,143,198,252–257], clinoptilolite [134,138], and sodium bentonite [158,258]. Antioxidants like butylated hydroxytolouene (BHT) [137,259–263] and turmeric [124,264] can also mitigate the severity of aflatoxicosis.

6.3. Probiotics

Many Gram-positive bacteria, including *Streptococcus*, *Enterococcus*, *Lactococcus*, and *Brevibacillus*, can bind AFB₁ *in vitro* [265–269]. However, most research has focused on probiotic strains of *Lactobacillus*, *Bifidobacterium*, and *Propionibacterium* [42,266,267,270–288]. Interactions between AFB₁ and *Lactobacillus rhamnosus* GG (LGG), *L. rhamnosus* LC-705 (LC-705), *Propionibacterium freudenreichii* strain *shermanii* JS (PJS) or mixtures of these strains have been shown to be especially effective [42,270–274,276–280,284–286,288]. A mixture of these strains was also utilized in our transcriptomic analyzes [238,240]. As gastrointestinal commensals or cultures used in cheese-making, yogurt and other dairy products, the safety of these lactic acid bacterial strains is well-established and easily applicable as potential chemopreventatives.

Strains LGG and LC-705 can sequester up to 80% of AFB₁ introduced into growth media [272,280]. AFB₁ interacts with the thick peptidoglycan layer characteristic of the Gram-positive bacterial cell wall [282,285]. When bound to AFB₁ *in vitro*, LGG and a mixture of LC-705 and PJS interacted less with intestinal mucus [276]. Incubation of LGG with AFB₁ reduced LGG adhesion to a Caco-2 intestinal cell monolayer [284] and decreased transport of AFB₁ across the monolayer [278]. These

in vitro models suggest the probiotic and the toxin would be excreted together *in vivo* and thereby decrease the effective dose of AFB₁.

Reduced AFB₁ toxicity has been demonstrated in both mice [270] and in rats [277] after addition of dietary LGG. In humans, probiotics enhanced excretion of AFB₁ [289,290]. Furthermore, addition of LGG, LC-705 or PJS *ex vivo* through injection of AFB₁ into the lumen of the chicken duodenum significantly reduced AFB₁ uptake (by 74%, 63% and 37%, respectively) [274]. A mixture of LC-705 and PJS caused a 40% reduction in AFB₁ absorption into chicken duodenal tissue in a repeat experiment [42]. Therefore, a probiotic mixture including LGG or LC-705 could be an effective preventative for aflatoxicosis if added to poultry feeds.

6.4. Selection for Resistance

Another option to minimize the adverse effects of AFB₁ is to increase the resistance of domestic poultry [7]. Selection for AFB₁ resistant lines of chicken [291–295] and quail [296–299] has been investigated; however, selection studies have not been performed in the far more sensitive domestic turkey. Improvement in AFB₁ resistance is highly dependent on starting population [299] and selection is most effective during AFB₁ challenge since correlations of phenotypic measures like weight gain or blood parameters are most informative during exposure [291,292,298]. The requirement for concurrent aflatoxicosis makes phenotypic selection difficult to implement in a commercial setting. However, understanding the molecular mechanism of aflatoxicosis in domestic turkey and identifying the genetic differences underlying decreased sensitivity in wild turkeys could allow targeted genetic selection for resistant alleles without constant AFB₁-exposure. Due to the similarity of symptoms of aflatoxicosis across species, potential genetic targets may be translatable to other poultry species.

7. Conclusions: Suggested Areas for Further Research

Future gene expression analyzes can provide insight into the mechanisms of aflatoxicosis and methods to reduce its effects. Our characterization of AFB₁-induced changes in the turkey liver and spleen transcriptomes identified genes responding to aflatoxicosis and host responses to toxicity. Since both proliferation and apoptosis occur during aflatoxicosis, the expression of cell cycle genes in the liver needs to be quantified alongside measures of apoptotic and mitotic cells. Similarly, gene expression in immune tissues such as the spleen should be measured concurrent with lymphocyte numbers, activation or apoptotic state to better clarify gene functions. Investigation of individual cell types could also detect cell-specific gene modulation. For example, the effects of AFB₁ on expression of immune genes could be measured in heterophils or T lymphocyte subsets (e.g., CD4⁺ versus CD8⁺ T cells). Gene expression in cells of the bile duct, known targets of AFB₁ mutagenesis, could be used to characterize hyperplasia. Expression patterns, as determined from mRNA, do not always directly correlate with protein levels or stability; therefore, proteomics could confirm effects on cell cycle regulators or immune mediators and provide a measure for AFB₁ inhibition of protein synthesis.

Analyses of systemic responses to AFB₁ in other tissues, such as the bursa, thymus, kidney or small intestine are needed to fully elucidate AFB₁ effects. Intestinal epithelial cells are directly exposed to AFB₁ during absorption and the potential prevention of AFB₁ uptake by feed additives also occurs within the small intestine. Therefore, investigation of intestinal transcriptome responses to AFB₁ is a priority.

The utility of a *Lactobacillus*-based probiotics as preventative for aflatoxicosis requires further examination *in vivo*. Higher concentrations or different compositions of dietary probiotics should be examined for their ability to protect poultry from the development of hepatic lesions and in restoring gene expression profiles. Furthermore, no definitive conclusions can be made regarding the effects of probiotics without characterizing the intestinal microbiota. Shifts in bacterial population structure of the microbiome could be investigated by 16S NGS sequencing for direct comparison to the host effects shown by RNA-seq.

Comparative analysis of transcriptome responses of poultry to AFB₁ could help resolve differences in their sensitivity. RNA-seq studies investigating both domestic and wild turkey using an *in ovo* exposure model [300] and dietary challenge of poults [301] are currently underway. Preliminary data from embryonic exposures illustrates conserved effects on cell cycle regulators and variation in metabolic and anti-oxidant enzymes [300]. Genes and pathways identified in these studies will provide targets for selection efforts to improve resistance to aflatoxicosis in domestic poultry.

Acknowledgments

The authors gratefully acknowledge support from the United States Department of Agriculture, Agriculture and Food Research Initiative Grants 2005-01326, 2007-35205-17880, 2009-35205-05302, and 2013-01043 from the USDA National Institute of Food and Agriculture Animal Genome Program. Support from the Minnesota and Utah Agricultural Experiments Stations is also acknowledged.

Author Contributions

The work presented is a part of a Ph.D. thesis; the information was collected and summarized by Melissa Monson who took the lead on writing the manuscript. Kent Reed and Roger Coulombe supervised the work and edited the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Bedard, L.L.; Massey, T.E. Aflatoxin B₁-induced DNA damage and its repair. *Cancer Lett.* **2006**, *241*, 174–183.
2. Bennett, J.W.; Klich, M. Mycotoxins. *Clin. Microbiol. Rev.* **2003**, *16*, 497–516.
3. Coulombe, R.A., Jr. Biological action of mycotoxins. *J. Dairy Sci.* **1993**, *76*, 880–891.
4. Eaton, D.L.; Gallagher, E.P. Mechanisms of aflatoxin carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 135–172.
5. Groopman, J.D.; Kensler, T.W. Role of metabolism and viruses in aflatoxin-induced liver cancer. *Toxicol. Appl. Pharmacol.* **2005**, *206*, 131–137.
6. Kensler, T.W.; Roebuck, B.D.; Wogan, G.N.; Groopman, J.D. Aflatoxin: A 50-Year odyssey of mechanistic and translational toxicology. *Toxicol. Sci.* **2011**, *120*, S28–S48.

7. Leeson, S.; Diaz, G.J.; Summers, J.D. Aflatoxins. In *Poultry Metabolic Disorders and Mycotoxins*; University Books: Guelph, Canada, 1995.
8. Yabe, K.; Nakajima, H. Enzyme reactions and genes in aflatoxin biosynthesis. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 745–755.
9. Wogan, G.N.; Kensler, T.W.; Groopman, J.D. Present and future directions of translational research on aflatoxin and hepatocellular carcinoma. A review. *Food Addit. Contam.* **2012**, *29*, 249–257.
10. Newberne, P.M.; Butler, W.H. Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals: A review. *Cancer Res.* **1969**, *29*, 236–250.
11. Patterson, D.S.P. Metabolism as a factor in determining the toxic action of the aflatoxins in different animal species. *Food Cosmet. Toxicol.* **1973**, *11*, 287–294.
12. Pier, A.C. Aflatoxicosis and Immunosuppression in Mammalian Animals. In *Aflatoxin in Maize: A Proceedings of the Workshop*; Zuber, M.S., Lillehoj, E.B., Renfro, B.L., Eds.; CIMMYT: El Batan, Mexico, 1986.
13. Robens, J.F.; Richard, J.L. Aflatoxins in animal and human health. *Rev. Environ. Contam. Toxicol.* **1992**, *127*, 69–94.
14. Wogan, G.N. Chemical nature and biological effects of the aflatoxins. *Bacteriol. Rev.* **1966**, *30*, 460–470.
15. Wogan, G.N. Aflatoxins as risk factors for hepatocellular carcinoma in humans. *Cancer Res.* **1992**, *52*, 2114s–2118s.
16. Blount, W.P. Turkey “X” disease. *Turkeys* **1961**, *9*, 77.
17. Wannop, C.C. The histopathology of turkey “X” disease in Great Britain. *Avian Dis.* **1961**, *5*, 371–381.
18. Nesbitt, B.F.; O’Kelly, J.; Sargeant, K.; Sheridan, A. *Aspergillus flavus* and turkey X disease. Toxic metabolites of *Aspergillus flavus*. *Nature* **1962**, *195*, 1062–1063.
19. Cary, J.W.; Klich, M.A.; Beltz, S.B. Characterization of aflatoxin-producing fungi outside of *Aspergillus* section *Flavi*. *Mycologia* **2005**, *97*, 425–432.
20. Frisvad, J.C.; Skouboe, P.; Samson, R.A. Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B₁, sterigmatocystin and 3-O-methylsterigmatocystin, *Aspergillus rambellii* sp. nov. *Syst. Appl. Microbiol.* **2005**, *28*, 442–453.
21. Rawal, S.; Kim, J.E.; Coulombe, R. Aflatoxin B₁ in poultry: Toxicology, metabolism and prevention. *Res. Vet. Sci.* **2010**, *89*, 325–331.
22. Cullen, J.M.; Newberne, P.M. Acute Hepatotoxicity of Aflatoxins. In *The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance*; Eaton, D.L., Groopman, J.D., Eds.; Academic Press: London, UK, 1993.
23. CAST. *Mycotoxins: Risks in Plant, Animal and Human Systems*; No. 139. Council for Agricultural Science and Technology: Ames, IA, USA, 2003.
24. Cotty, P.J.; Jaime-Garcia, R. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *Int. J. Food Microbiol.* **2007**, *119*, 109–115.

25. Gqaleni, N.; Smith, J.E.; Lacey, J.; Gettinby, G. Effects of temperature, water activity, and incubation time on production of aflatoxins and cyclopiazonic acid by an isolate of *Aspergillus flavus* in surface agar culture. *Appl. Environ. Microbiol.* **1997**, *63*, 1048–1053.
26. Medina A.; Rodriguez A.; Magan N. Effect of climate change on *Aspergillus flavus* and aflatoxin B₁ production. *Front. Microbiol.* **2014**, *5*, 348.
27. Mousa, W.; Ghazali, F.M.; Jinap, S.; Ghazali, H.M.; Radu, S. Modeling growth rate and assessing aflatoxins production by *Aspergillus flavus* as a function of water activity and temperature on polished and brown rice. *J. Food Sci.* **2013**, *78*, M56–M63.
28. Schindler, A.F.; Palmer, J.G.; Eisenberg, W.V. Aflatoxin production by *Aspergillus flavus* as related to various temperatures. *Appl. Microbiol.* **1967**, *15*, 1006–1009.
29. Schroeder, H.W.; Hein, H.. Aflatoxins: Production of the toxins *in vitro* in relation to temperature. *Appl. Microbiol.* **1967**, *15*, 441–445.
30. Trenk, H.L.; Hartman, P.A. Effects of moisture content and temperature on aflatoxin production in corn. *Appl. Microbiol.* **1970**, *19*, 781–784.
31. Aly, S.A.; Anwer, W. Effect of naturally contaminated feed with aflatoxins on performance of laying hens and the carryover of aflatoxin B₁ residues in table egg. *Pakistan J. Nutr.* **2009**, *8*, 181–186.
32. Chen, C.; Pearson, A.M.; Coleman, T.H.; Gray, J.I.; Pestka, J.J.; Aust, S.D. Tissue deposition and clearance of aflatoxins from broiler chickens fed a contaminated diet. *Food Chem. Toxicol.* **1984**, *22*, 447–451.
33. Galvano, F.; Galofaro, V.; Galvano, G. Occurrence and stability of aflatoxin M₁ in milk and milk products: A worldwide review. *J. Food Prot.* **1996**, *59*, 1079–1090.
34. Pandey, I.; Chauhan, S.S. Studies on production performance and toxin residues in tissues and eggs of layer chickens fed on diets with various concentrations of aflatoxin AFB₁. *Br. Poult. Sci.* **2007**, *48*, 713–723.
35. Purchase, I.F.H. Aflatoxin residues in food of animal origin. *Food Cosmet. Toxicol.* **1972**, *10*, 531–544.
36. Richard, J.L.; Stubblefield, R.D.; Lyon, R.L.; Peden, W.M.; Thurston, J.R.; Rimler, R.B. Distribution and clearance of aflatoxins B₁ and M₁ in turkeys fed diets containing 50 or 150 ppb aflatoxin from naturally contaminated corn. *Avian Dis.* **1986**, *30*, 788–793.
37. Wolzak, A.; Pearson, A.M.; Coleman, T.H.; Pestka, J.J.; Gray, J.I. Aflatoxin deposition and clearance in the eggs of laying hens. *Food Chem. Toxicol.* **1985**, *23*, 1057–1061.
38. Wolzak, A.; Pearson, A.M.; Coleman, T.H.; Pestka, J.J.; Gray, J.I.; Chen, C. Aflatoxin carryover and clearance from tissues of laying hens. *Food Chem. Toxicol.* **1986**, *24*, 37–41.
39. Giambrone, J.J.; Diener, U.L.; Davis, N.D.; Panangala, V.S.; Hoerr, F.J. Effects of aflatoxin on young turkeys and broiler chickens. *Poult. Sci.* **1985**, *64*, 1678–1684.
40. Klein, P.J.; Buckner, R.; Kelly, J.; Coulombe, R.A., Jr. Biochemical basis for the extreme sensitivity of turkeys to aflatoxin B₁. *Toxicol. Appl. Pharmacol.* **2000**, *165*, 45–52.
41. CAST. *Mycotoxins: Economic and Health Risks*; No. 116. Council for Agricultural Science and Technology: Ames, IA, USA, 1989.
42. Gratz, S.; Mykkänen, H.; el-Nezami, H. Aflatoxin B₁ binding by a mixture of *Lactobacillus* and *Propionibacterium*.: *In vitro* versus *ex vivo*. *J. Food Prot.* **2005**, *68*, 2470–2474.

43. Guengerich, F.P.; Johnson, W.W.; Ueng, Y.F.; Yamazaki, H.; Shimada, T. Involvement of cytochrome P450, glutathione *S*-transferase, and epoxide hydrolase in the metabolism of aflatoxin B₁ and relevance to risk of human liver cancer. *Environ. Health Perspect.* **1996**, *104*, 557–562.
44. Godoy, H.M.; Neal, G.E. Some studies of the effects of aflatoxin B₁ *in vivo* and *in vitro* on nucleic acid synthesis in rat and mouse. *Chem. Biol. Interact.* **1976**, *13*, 257–277.
45. Monroe, D.H.; Eaton, D.L. Comparative effects of butylated hydroxyanisole on hepatic *in vivo* DNA binding and *in vitro* biotransformation of aflatoxin B₁ in the rat and mouse. *Toxicol. Appl. Pharmacol.* **1987**, *90*, 401–409.
46. Pelkonen, P.; Lang, M.A.; Wild, C.P.; Negishi, M.; Juvonen, R.O. Activation of aflatoxin B₁ by mouse CYP2A enzymes and cytotoxicity in recombinant yeast cells. *Eur. J. Pharmacol.* **1994**, *292*, 67–73.
47. Pelkonen, P.; Lang, M.A.; Negishi, M.; Wild, C.P.; Juvonen, R.O. Interaction of aflatoxin B₁ with cytochrome P450 2A5 and its mutants: Correlation with metabolic activation and toxicity. *Chem. Res. Toxicol.* **1997**, *10*, 85–90.
48. Ramsdell, H.S.; Eaton, D.L. Species susceptibility to aflatoxin B₁ carcinogenesis: Comparative kinetics of microsomal biotransformation. *Cancer Res.* **1990**, *50*, 615–620.
49. Yanagimoto, T.; Itoh, S.; Sawada, M.; Hashimoto, H.; Kamataki, T. Molecular cloning and functional expression of a mouse cytochrome *P*-450 (Cyp3a-13): Examination of Cyp3a-13 enzyme to activate aflatoxin B₁ (AFB₁). *Biochim. Biophys. Acta* **1994**, *1201*, 405–410.
50. Yanagimoto, T.; Itoh, S.; Sawada, M.; Kamataki, T. Mouse cytochrome P450 (Cyp3a11): Predominant expression in liver and capacity to activate aflatoxin B₁. *Arch. Biochem. Biophys.* **1997**, *340*, 215–218.
51. Buetler, T.M.; Slone, D.; Eaton, D.L. Comparison of the aflatoxin B₁-8,9-epoxide conjugating activities of two bacterially expressed alpha class glutathione *S*-transferase isozymes from mouse and rat. *Biochem. Biophys. Res. Commun.* **1992**, *188*, 597–603.
52. Hayes, J.D.; Judah, D.J.; Neal, G.E.; Nguyen, T. Molecular cloning and heterologous expression of a cDNA encoding a mouse glutathione *S*-transferase Yc subunit possessing high catalytic activity for aflatoxin B₁-8,9-epoxide. *Biochem. J.* **1992**, *285*, 173–180.
53. Ilic, Z.; Crawford, D.; Vakharia, D.; Egner, P.A.; Sell, S. Glutathione-*S*-transferase A3 knockout mice are sensitive to acute cytotoxic and genotoxic effects of aflatoxin B₁. *Toxicol. Appl. Pharmacol.* **2010**, *242*, 241–246.
54. Monroe, D.H.; Eaton, D.L. Effects of modulation of hepatic glutathione on biotransformation and covalent binding of aflatoxin B₁ to DNA in the mouse. *Toxicol. Appl. Pharmacol.* **1988**, *94*, 118–127.
55. Quinn, B.A.; Crane, T.L.; Kocal, T.E.; Best, S.J.; Cameron, R.G.; Rushmore, T.H.; Farber, E.; Hayes, M.A. Protective activity of different hepatic cytosolic glutathione *S*-transferases against DNA-binding metabolites of aflatoxin B₁. *Toxicol. Appl. Pharmacol.* **1990**, *105*, 351–363.
56. Slone, D.H.; Gallagher, E.P.; Ramsdell, H.S.; Rettie, A.E.; Stapleton, P.L.; Berlad, L.G.; Eaton, D.L. Human variability in hepatic glutathione *S*-transferase-mediated conjugation of aflatoxin B₁-epoxide and other substrates. *Pharmacogenetics* **1995**, *5*, 224–233.

57. Buetler, T.M.; Bammler, T.K.; Hayes, J.D.; Eaton, D.L. Oltipraz-mediated changes in aflatoxin B₁ biotransformation in rat liver: Implications for human chemoprevention. *Cancer Res.* **1996**, *56*, 2306–2313.
58. Imaoka, S.; Ikemoto, S.; Shimada, T.; Funae, Y. Mutagenic activation of aflatoxin B₁ by pulmonary, renal, and hepatic cytochrome P450s from rats. *Mutat. Res.* **1992**, *269*, 231–236.
59. Johnson, W.W.; Ueng, Y.F.; Widersten, M.; Mannervik, B.; Hayes, J.D.; Sherratt, P.J.; Ketterer, B.; Guengerich, F.P. Conjugation of highly reactive aflatoxin B₁ *exo*-8,9-epoxide catalyzed by rat and human glutathione transferases: Estimation of kinetic parameters. *Biochemistry* **1997**, *36*, 3056–3060.
60. Hayes, J.D.; Judah, D.J.; McLellan, L.I.; Kerr, L.A.; Peacock, S.D.; Neal, G.E. Ethoxyquin-induced resistance to aflatoxin B₁ in the rat is associated with the expression of a novel alpha-class glutathione *S*-transferase subunit, Yc2, which possesses high catalytic activity for aflatoxin B₁-8,9-epoxide. *Biochem. J.* **1991**, *279*, 385–398.
61. Hayes, J.D.; Nguyen, T.; Judah, D.J.; Petersson, D.G.; Neal, G.E. Cloning of cDNAs from fetal rat liver encoding glutathione *S*-transferase Yc polypeptides. The Yc2 subunit is expressed in adult rat liver resistant to the hepatocarcinogen aflatoxin B₁. *J. Biol. Chem.* **1994**, *269*, 20707–20717.
62. Hayes, J.D.; Pulford, D.J.; Ellis, E.M.; McLeod, R.; James, R.F.; Seidegård, J.; Mosialou, E.; Jernström, B.; Neal, G.E. Regulation of rat glutathione *S*-transferase A5 by cancer chemopreventive agents: Mechanisms of inducible resistance to aflatoxin B₁. *Chem. Biol. Interact.* **1998**, *111–112*, 51–67.
63. Forrester, L.M.; Neal, G.E.; Judah, D.J.; Glancey, M.J.; Wolf, C.R. Evidence for involvement of multiple forms of cytochrome P-450 in aflatoxin B₁ metabolism in human liver. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 8306–8310.
64. Gallagher, E.P.; Wienkers, L.C.; Stapleton, P.L.; Kunze, K.L.; Eaton, D.L. Role of human microsomal and human complementary DNA-expressed cytochromes P4501A2 and P4503A4 in the bioactivation of aflatoxin B₁. *Cancer Res.* **1994**, *54*, 101–108.
65. Macé, K.; Aguilar, F.; Wang, J.S.; Vautravers, P.; Gómez-Lechón, M.; Gonzalez, F.J.; Groopman, J.; Harris, C.C.; Pfeifer, A.M. Aflatoxin B₁-induced DNA adduct formation and p53 mutations in CYP450-expressing human liver cell lines. *Carcinogenesis* **1997**, *18*, 1291–1297.
66. Ramsdell, H.S.; Parkinson, A.; Eddy, A.C.; Eaton, D.L. Bioactivation of aflatoxin B₁ by human liver microsomes: Role of cytochrome P450 IIIA enzymes. *Toxicol. Appl. Pharmacol.* **1991**, *108*, 436–447.
67. Van Vleet, T.R.; Watterson, T.L.; Klein, P.J.; Coulombe, R.A., Jr. Aflatoxin B₁ alters the expression of p53 in cytochrome P450-expressing human lung cells. *Toxicol. Sci.* **2006**, *89*, 399–407.
68. Raney, K.D.; Meyer, D.J.; Ketterer, B.; Harris, T.M.; Guengerich, F.P. Glutathione conjugation of aflatoxin B₁ *exo*- and *endo*-epoxides by rat and human glutathione *S*-transferases. *Chem. Res. Toxicol.* **1992**, *5*, 470–478.
69. Gross-Steinmeyer, K.; Stapleton, P.L.; Tracy, J.H.; Bammler, T.K.; Strom, S.C.; Eaton, D.L. Sulforaphane- and phenethyl isothiocyanate-induced inhibition of aflatoxin B₁-mediated genotoxicity

- in human hepatocytes: Role of *GSTM1* genotype and *CYP3A4* gene expression. *Toxicol. Sci.* **2010**, *116*, 422–432.
70. Liu, Y.H.; Taylor, J.; Linko, P.; Lucier, G.W.; Thompson, C.L. Glutathione *S*-transferase mu in human lymphocyte and liver: Role in modulating formation of carcinogen-derived DNA adducts. *Carcinogenesis* **1991**, *12*, 2269–2275.
 71. McGlynn, K.A.; Rosvold, E.A.; Lustbader, E.D.; Hu, Y.; Clapper, M.L.; Zhou, T.; Wild, C.P.; Xia, X.L.; Baffoe-Bonnie, A.; Ofori-Adjei, D.; *et al.* Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B₁. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 2384–2387.
 72. Arafa, A.S.; Bloomer, R.J.; Wilson, H.R.; Simpson, C.F.; Harms, R.H. Susceptibility of various poultry species to dietary aflatoxin. *Br. Poult. Sci.* **1981**, *22*, 431–436.
 73. Gumbmann, M.R.; Williams, S.N.; Booth, A.N.; Vohra, P.; Ernst, R.A.; Bethard, M. Aflatoxin susceptibility in various breeds of poultry. *Proc. Soc. Exp. Biol. Med.* **1970**, *134*, 683–688.
 74. Lozano, M.C.; Diaz, G.J. Microsomal and cytosolic biotransformation of aflatoxin B₁ in four poultry species. *Br. Poult. Sci.* **2006**, *47*, 734–741.
 75. Muller, R.D.; Carlson, C.W.; Semeniuk, G.; Harshfield, G.S. The response of chicks, ducklings, goslings, pheasants and poults to graded levels of aflatoxins. *Poult. Sci.* **1970**, *49*, 1346–1350.
 76. Rawal, S.; Mendoza, K.M.; Reed, K.M.; Coulombe, R.A. Jr. Structure, genetic mapping, and function of the cytochrome P450 3A37 gene in the turkey (*Meleagris gallopavo*). *Cytogenet. Genome Res.* **2009**, *125*, 67–73.
 77. Rawal, S.; Yip, S.S.; Coulombe, R.A., Jr. Cloning, expression and functional characterization of cytochrome P450 3A37 from turkey liver with high aflatoxin B₁ epoxidation activity. *Chem. Res. Toxicol.* **2010**, *23*, 1322–1329.
 78. Rawal, S.; Coulombe, R.A., Jr. Metabolism of aflatoxin B₁ in turkey liver microsomes: The relative roles of cytochromes P450 1A5 and 3A37. *Toxicol. Appl. Pharmacol.* **2011**, *254*, 349–354.
 79. Yip, S.S.; Coulombe, R.A. Molecular cloning and expression of a novel cytochrome P450 from turkey liver with aflatoxin B₁ oxidizing activity. *Chem. Res. Toxicol.* **2006**, *19*, 30–37.
 80. Diaz, G.J.; Murcia, H.W.; Cepeda, S.M. Bioactivation of aflatoxin B₁ by turkey liver microsomes: Responsible cytochrome P450 enzymes. *Br. Poult. Sci.* **2010**, *51*, 828–837.
 81. Diaz, G.J.; Murcia, H.W.; Cepeda, S.M. Cytochrome P450 enzymes involved in the metabolism of aflatoxin B₁ in chickens and quail. *Poult. Sci.* **2010**, *89*, 2461–2469.
 82. Diaz, G.J.; Murcia, H.W.; Cepeda, S.M.; Boermans, H.J. The role of selected cytochrome P450 enzymes on the bioactivation of aflatoxin B₁ by duck liver microsomes. *Avian Pathol.* **2010**, *39*, 279–285.
 83. Murcia, H.W.; Díaz, G.J.; Cepeda, S.M. Enzymatic activity in turkey, duck, quail and chicken liver microsomes against four human cytochrome P450 prototype substrates and aflatoxin B₁. *J. Xenobiotics* **2011**, *1*, 17–21.
 84. Giambone, J.J.; Diener, U.L.; Davis, N.D.; Panangala, V.S.; Hoerr, F.J. Effects of purified aflatoxin on turkeys. *Poult. Sci.* **1985**, *64*, 859–865.

85. Klein, P.J.; van Vleet, T.R.; Hall, J.O.; Coulombe, R.A., Jr. Biochemical factors underlying the age-related sensitivity of turkeys to aflatoxin B₁. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2002**, *132*, 193–201.
86. Hussain, Z.; Khan, M.Z.; Khan, A.; Javed, I.; Saleemi, M.K.; Mahmood, S.; Asi, M.R. Residues of aflatoxin B₁ in broiler meat: Effect of age and dietary aflatoxin B₁ levels. *Food Chem. Toxicol.* **2010**, *48*, 3304–3307.
87. Lanza, G.M.; Washburn, K.W.; Wyatt, R.D. Variation with age in response of broilers to aflatoxin. *Poult. Sci.* **1980**, *59*, 282–288.
88. Quezada, T.; Cuéllar, H.; Jaramillo-Juárez, F.; Valdivia, A.G.; Reyes, J.L. Effects of aflatoxin B₁ on the liver and kidney of broiler chickens during development. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2000**, *125*, 265–272.
89. Barraud, L.; Douki, T.; Guerret, S.; Chevallier, M.; Jamard, C.; Trepo, C.; Wild, C.P.; Cadet, J.; Cova, L. The role of duck hepatitis B virus and aflatoxin B₁ in the induction of oxidative stress in the liver. *Cancer Detect. Prev.* **2001**, *25*, 192–201.
90. Kim, J.E.; Bauer, M.M.; Mendoza, K.M.; Reed, K.M.; Coulombe, R.A., Jr. Comparative genomics identifies new alpha class genes within the avian glutathione *S*-transferase gene cluster. *Gene* **2010**, *452*, 45–53.
91. Kim, J.E.; Bunderson, B.R.; Croasdell, A.; Coulombe, R.A., Jr. Functional characterization of alpha-class glutathione *S*-transferases from the turkey (*Meleagris gallopavo*). *Toxicol. Sci.* **2011**, *124*, 45–53.
92. Quist, C.F.; Bounous, D.I.; Kilburn, J.V.; Nettles, V.F.; Wyatt, R.D. The effect of dietary aflatoxin on wild turkey poults. *J. Wildl. Dis.* **2000**, *36*, 436–444.
93. Reed, K.M. Turkey. In *Genome Mapping and Genomics in Domestic Animals*; Cockett, N.E., Kole, C., Eds.; Springer-Verlag: Berlin, Germany, 2009.
94. Julian, R.J. Production and growth related disorders and other metabolic diseases of poultry—A review. *Vet. J.* **2005**, *169*, 350–369.
95. Bayyari, G.R.; Huff, W.E.; Rath, N.C.; Balog, J.M.; Newberry, L.A.; Villines, J.D.; Skeeles, J.K.; Anthony, N.B.; Nestor, K.E. Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. *Poult. Sci.* **1997**, *76*, 289–296.
96. Li, Z.; Nestor, K.E.; Saif, Y.M.; Luhtala, M. Flow cytometric analysis of T lymphocyte subpopulations in large-bodied turkey lines and a randombred control population. *Poult. Sci.* **2000**, *79*, 219–223.
97. Li, Z.; Nestor, K.E.; Saif, Y.M.; Anderson, J.W.; Patterson, R.A. Effect of selection for increased body weight in turkeys on lymphoid organ weights, phagocytosis, and antibody responses to fowl cholera and Newcastle disease-inactivated vaccines. *Poult. Sci.* **2001**, *80*, 689–694.
98. Van der Most, P.J.; de Jong, B.; Parmentier, H.K.; Verhulst, S. Trade-off between growth and immune function: A meta-analysis of selection experiments. *Funct. Ecol.* **2011**, *25*, 74–80.
99. Kim, J.E.; Bunderson, B.R.; Croasdell, A.; Reed, K.M.; Coulombe, R.A., Jr. Alpha-class glutathione *S*-transferases in wild turkeys (*Meleagris gallopavo*): Characterization and role in resistance to the carcinogenic mycotoxin aflatoxin B₁. *PLoS ONE* **2013**, *8*, e60662.

100. Bunderson, B.R.; Kim, J.E.; Croasdell, A.; Mendoza, K.M.; Reed, K.M.; Coulombe, R.A., Jr. Heterologous expression and functional characterization of avian mu-class glutathione S-transferases. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2013**, *158*, 109–116.
101. Smela, M.E.; Hamm, M.L.; Henderson, P.T.; Harris, C.M.; Harris, T.M.; Essigmann, J.M. The aflatoxin B₁ formamidopyrimidine adduct plays a major role in causing the types of mutations observed in human hepatocellular carcinoma. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 6655–6660.
102. Corrier, D.E. Mycotoxicosis: Mechanisms of immunosuppression. *Vet. Immunol. Immunopathol.* **1991**, *30*, 73–87.
103. Guengerich, F.P.; Arneson, K.O.; Williams, K.M.; Deng, Z.; Harris, T.M. Reaction of aflatoxin B₁ oxidation products with lysine. *Chem. Res. Toxicol.* **2002**, *15*, 780–792.
104. Iwaki, M.; Kitagawa, T.; Akamatsu, Y.; Aibara, K. Cytotoxic effects of aflatoxin B₁ and its association with cellular components in chicken embryo primary cultured cells. *Biochim. Biophys. Acta* **1990**, *1035*, 146–153.
105. IARC. Aflatoxins. In *A Review of Human Carcinogens. Part. F: Chemical Agents and Related Occupations*; International Agency for Research on Cancer Working Group on the Evaluation of Carcinogenic Risks to Humans: Lyon, France, 2012.
106. Aguilar, F.; Hussain, S.P.; Cerutti, P. Aflatoxin B₁ induces the transversion of G→T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 8586–8590.
107. Shen, H.M.; Ong, C.N. Mutations of the p53 tumor suppressor gene and *ras* oncogenes in aflatoxin hepatocarcinogenesis. *Mutat. Res.* **1996**, *366*, 23–44.
108. Wild, C.P.; Montesano, R. A model of interaction: Aflatoxins and hepatitis viruses in liver cancer aetiology and prevention. *Cancer Lett.* **2009**, *286*, 22–28.
109. Butler, W.H.; Greenblatt, M.; Lijinsky, W. Carcinogenesis in rats by aflatoxins B₁, G₁, and B₂. *Cancer Res.* **1969**, *29*, 2206–2211.
110. Wogan, G.N.; Newberne, P.M. Dose-response characteristics of aflatoxin B₁ carcinogenesis in the rat. *Cancer Res.* **1967**, *27*, 2370–2376.
111. Wogan, G.N.; Paglialunga, S.; Newberne, P.M. Carcinogenic effects of low dietary levels of aflatoxin B₁ in rats. *Food Cosmet. Toxicol.* **1974**, *12*, 681–685.
112. Cova, L.; Wild, C.P.; Mehrotra, R.; Turusov, V.; Shirai, T.; Lambert, V.; Jacquet, C.; Tomatis, L.; Trépo, C.; Montesano, R. Contribution of aflatoxin B₁ and hepatitis B virus infection in the induction of liver tumors in ducks. *Cancer Res.* **1990**, *50*, 2156–2163.
113. Cullen, J.M.; Marion, P.L.; Sherman, G.J.; Hong, X.; Newbold, J.E. Hepatic neoplasms in aflatoxin B₁-treated, congenital duck hepatitis B virus-infected, and virus-free Pekin ducks. *Cancer Res.* **1990**, *50*, 4072–4080.
114. Uchida, T.; Suzuki, K.; Esumi, M.; Arai, M.; Shikata, T. Influence of aflatoxin B₁ intoxication on duck livers with duck hepatitis B virus infection. *Cancer Res.* **1988**, *48*, 1559–1565.
115. Chen, X.; Horn, N.; Applegate, T.J. Efficiency of hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of graded levels of aflatoxin B₁ in broiler chicks. *Poult. Sci.* **2014**, *93*, 2037–2047.
116. Giambone, J.J.; Diener, U.L.; Davis, N.D.; Panangala, V.S.; Hoerr, F.J. Effects of purified aflatoxin on broiler chickens. *Poult. Sci.* **1985**, *64*, 852–858.

117. Huff, W.E.; Kubena, L.F.; Harvey, R.B.; Carrier, D.E.; Mollenhauer, H.H. Progression of aflatoxicosis in broiler chickens. *Poult. Sci.* **1986**, *65*, 1891–1899.
118. Rauber, R.H.; Dilkin, P.; Giacomini, L.Z.; Araújo de Almeida, C.A.; Mallmann, C.A. Performance of turkey poults fed different doses of aflatoxins in the diet. *Poult. Sci.* **2007**, *86*, 1620–1624.
119. Richard, J.L.; Pier, A.C.; Cysewski, S.J.; Graham, C.K. Effect of aflatoxin and aspergillosis on turkey poults. *Avian Dis.* **1973**, *17*, 111–121.
120. Smith, J.W.; Hamilton, P.B. Aflatoxicosis in the broiler chicken. *Poult. Sci.* **1970**, *49*, 207–215.
121. Sims, W.M., Jr.; Kelley, D.C.; Sanford, P.E. A study of aflatoxicosis in laying hens. *Poult. Sci.* **1970**, *49*, 1082–1084.
122. Verma, J.; Johri, T.S.; Swain, B.K.; Ameena, S. Effect of graded levels of aflatoxin, ochratoxin and their combinations on the performance and immune response of broilers. *Br. Poult. Sci.* **2004**, *45*, 512–518.
123. Yarru, L.P.; Settivari, R.S.; Antoniou, E.; Ledoux, D.R.; Rottinghaus, G.E. Toxicological and gene expression analysis of the impact of aflatoxin B1 on hepatic function of male broiler chicks. *Poult. Sci.* **2009**, *88*, 360–371.
124. Yarru, L.P.; Settivari, R.S.; Gowda, N.K.; Antoniou, E.; Ledoux, D.R.; Rottinghaus, G.E. Effects of turmeric (*Curcuma longa*) on the expression of hepatic genes associated with biotransformation, antioxidant, and immune systems in broiler chicks fed aflatoxin. *Poult. Sci.* **2009**, *88*, 2620–2627.
125. Lee, J.T.; Jessen, K.A.; Beltran, R.; Starkl, V.; Schatzmayr, G.; Borutova, R.; Caldwell, D.J. Mycotoxin-contaminated diets and deactivating compound in laying hens: 1. Effects on performance characteristics and relative organ weight. *Poult. Sci.* **2012**, *91*, 2089–2095.
126. Oliveira, C.A.; Rosmaninho, J.F.; Butkeraitis, P.; Corrêa, B.; Reis, T.A.; Guerra, J.L.; Albuquerque, R.; Moro, M.E. Effect of low levels of dietary aflatoxin B₁ on laying Japanese quail. *Poult. Sci.* **2002**, *81*, 976–980.
127. Chen, X.; Horn, N.; Cotter, P.F.; Applegate, T.J. Growth, serum biochemistry, complement activity, and liver gene expression responses of Pekin ducklings to graded levels of cultured aflatoxin B₁. *Poult. Sci.* **2014**, *93*, 2028–2036.
128. Azzam, A.H.; Gabal, M.A. Aflatoxin and immunity in layer hens. *Avian Pathol.* **1998**, *27*, 570–577.
129. Garlich, J.D.; Tung, H.T.; Hamilton, P.B. The effects of short term feeding of aflatoxin on egg production and some plasma constituents of the laying hen. *Poult. Sci.* **1973**, *52*, 2206–2211.
130. Howarth, B., Jr.; Wyatt, R.D. Effect of dietary aflatoxin on fertility, hatchability, and progeny performance of broiler breeder hens. *Appl. Environ. Microbiol.* **1976**, *31*, 680–684.
131. Khan, W.A.; Khan, M.Z.; Khan, A.; Hassan, Z.U.; Rafique, S.; Saleemi, M.K.; Ahad, A. Dietary vitamin E in White Leghorn layer breeder hens: A strategy to combat aflatoxin B₁-induced damage. *Avian Pathol.* **2014**, *43*, 389–395.
132. Lee, J.T.; Jessen, K.A.; Beltran, R.; Starkl, V.; Schatzmayr, G.; Borutova, R.; Caldwell, D.J. Effects of mycotoxin-contaminated diets and deactivating compound in laying hens: 2. Effects on white shell egg quality and characteristics. *Poult. Sci.* **2012**, *91*, 2096–2104.

133. Oliveira, C.A.F.; Rosmaninho, J.F.; Castro, A.L.; Butkeraitis, P.; Alves Reis, T.; Corrêa, B. Aflatoxin residues in eggs of laying Japanese quail after long-term administration of rations containing low levels of aflatoxin B₁. *Food Addit. Contam.* **2003**, *20*, 648–653.
134. Ortatatli, M.; Oğuz, H. Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis. *Res. Vet. Sci.* **2001**, *71*, 59–66.
135. Merkley, J.W.; Maxwell, R.J.; Phillips, J.G.; Huff, W.E. Hepatic fatty acid profiles in aflatoxin-exposed broiler chickens. *Poult. Sci.* **1987**, *66*, 59–67.
136. Siloto, E.V.; Oliveira, E.F.; Sartori, J.R.; Fascina, V.B.; Martins, B.A.; Ledoux, D.R.; Rottinghaus, G.E.; Sartori, D.R. Lipid metabolism of commercial layers fed diets containing aflatoxin, fumonisin, and a binder. *Poult. Sci.* **2013**, *92*, 2077–2083.
137. Klein, P.J.; van Vleet, T.R.; Hall, J.O.; Coulombe, R.A., Jr. Dietary butylated hydroxytoluene protects against aflatoxicosis in turkeys. *Toxicol. Appl. Pharmacol.* **2002**, *182*, 11–19.
138. Ortatatli, M.; Oğuz, H.; Hatipoğlu, F.; Karaman, M. Evaluation of pathological changes in broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure. *Res. Vet. Sci.* **2005**, *78*, 61–68.
139. Chen, K.; Fang, J.; Peng, X.; Cui, H.; Chen, J.; Wang, F.; Chen, Z.; Zuo, Z.; Deng, J.; Lai, W.; Zhou, Y. Effect of selenium supplementation on aflatoxin B₁-induced histopathological lesions and apoptosis in bursa of Fabricius in broilers. *Food Chem. Toxicol.* **2014**, *74*, 91–97.
140. Kubena, L.F.; Harvey, R.B.; Bailey, R.H.; Buckley, S.A.; Rottinghaus, G.E. Effects of a hydrated sodium calcium aluminosilicate (T-Bind) on mycotoxicosis in young broiler chickens. *Poult. Sci.* **1998**, *77*, 1502–1509.
141. Tung, H.T.; Wyatt, R.D.; Thaxton, P.; Hamilton, P.B. Concentrations of serum proteins during aflatoxicosis. *Toxicol. Appl. Pharmacol.* **1975**, *34*, 320–326.
142. Fernández, A.; Verde, M.T.; Gomez, J.; Gascon, M.; Ramos, J.J. Changes in the prothrombin time, haematology and serum proteins during experimental aflatoxicosis in hens and broiler chickens. *Res. Vet. Sci.* **1995**, *58*, 119–122.
143. Doerr, J.A.; Huff, W.E.; Tung, H.T.; Wyatt, R.D.; Hamilton, P.B. A survey of T-2 toxin, ochratoxin, and aflatoxin for their effects on the coagulation of blood in young broiler chickens. *Poult. Sci.* **1974**, *53*, 1728–1734.
144. Doerr, J.A.; Wyatt, R.D.; Hamilton, P.B. Impairment of coagulation function during aflatoxicosis in young chickens. *Toxicol. Appl. Pharmacol.* **1976**, *35*, 437–446.
145. Doerr, J.A.; Hamilton, P.B. Aflatoxicosis and intrinsic coagulation function in broiler chickens. *Poult. Sci.* **1981**, *60*, 1406–1411.
146. Witlock, D.R.; Wyatt, R.D. Effect of dietary aflatoxin on hemostasis of young turkey poults. *Poult. Sci.* **1981**, *60*, 528–531.
147. Donaldson, W.E.; Tung, H.T.; Hamilton, P.B. Depression of fatty acid synthesis in chick liver (*Gallus domesticus*) by aflatoxin. *Comp. Biochem. Physiol. B* **1972**, *41*, 843–847.
148. Hoerr, F.J. Clinical aspects of immunosuppression in poultry. *Avian Dis.* **2010**, *54*, 2–15.
149. Chen, K.; Shu, G.; Peng, X.; Fang, J.; Cui, H.; Chen, J.; Wang, F.; Chen, Z.; Zuo, Z.; Deng, J.; Geng, Y.; Lai, W. Protective role of sodium selenite on histopathological lesions decreased T-cell subsets and increased apoptosis of thymus in broilers intoxicated with aflatoxin B₁. *Food Chem. Toxicol.* **2013**, *59*, 446–454.

150. Chen, K.; Peng, X.; Rang, J.; Cui, H.; Zuo, Z.; Deng, J.; Chen, Z.; Geng, Y.; Lai, W.; Tang, L.; Yang, Q. Effects of dietary selenium on histopathological changes and T cells of spleen in broilers exposed to aflatoxin B₁. *Int. J. Environ. Res. Public Health* **2014**, *11*, 1904–1913.
151. Pier, A.C.; Heddleston, K.L.; Cysewski, S.J.; Patteron, J.M. Effect of aflatoxin on immunity in turkeys. II. Reversal of impaired resistance to bacterial infection by passive transfer of plasma. *Avian Dis.* **1972**, *16*, 381–387.
152. Thaxton, J.P.; Tung, H.T.; Hamilton, P.B. Immunosuppression in chickens by aflatoxin. *Poult. Sci.* **1974**, *53*, 721–725.
153. Edrington, T.S.; Kubena, L.F.; Harvey, R.B.; Rottinghaus, G.E. Influence of a superactivated charcoal on the toxic effects of aflatoxin or T-2 toxin in growing broilers. *Poult. Sci.* **1997**, *76*, 1205–1211.
154. Huff, W.E.; Kubena, L.F.; Harvey, R.B.; Hagler, W.M., Jr.; Swanson, S.P.; Phillips, T.D.; Creger, C.R. Individual and combined effects of aflatoxin and deoxynivalenol (DON, vomitoxin) in broiler chickens. *Poult. Sci.* **1986**, *65*, 1291–1298.
155. Huff, W.E.; Harvey, R.B.; Kubena, L.F.; Rottinghaus, G.E. Toxic synergism between aflatoxin and T-2 toxin in broiler chickens. *Poult. Sci.* **1988**, *67*, 1418–1423.
156. Peng, X.; Zhang, K.; Bai, S.; Ding, X.; Zeng, Q.; Yang, J.; Fang, J.; Chen, K. Histological lesions, cell cycle arrest, apoptosis and T cell subsets changes of spleen in chicken fed aflatoxin-contaminated corn. *Int. J. Environ. Res. Public Health* **2014**, *11*, 8567–8580.
157. Chang, C.F.; Hamilton, P.B. Impaired phagocytosis by heterophils from chickens during aflatoxicosis. *Toxicol. Appl. Pharmacol.* **1979**, *48*, 459–466.
158. Ibrahim, I.K.; Shareef, A.M.; Al-Joubory, K.M. Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. *Res. Vet. Sci.* **2000**, *69*, 119–122.
159. Ghosh, R.C.; Chauhan, H.V.; Jha, G.J. Suppression of cell-mediated immunity by purified aflatoxin B₁ in broiler chicks. *Vet. Immunol. Immunopathol.* **1991**, *28*, 165–172.
160. Neldon-Ortiz, D.L.; Qureshi, M.A. Effects of AFB₁ embryonic exposure on chicken mononuclear phagocytic cell functions. *Dev. Comp. Immunol.* **1992**, *16*, 187–196.
161. Qureshi, M.A.; Brake, J.; Hamilton, P.B.; Hagler, W.M., Jr.; Nesheim, S. Dietary exposure of broiler breeders to aflatoxin results in immune dysfunction in progeny chicks. *Poult. Sci.* **1998**, *77*, 812–819.
162. Chang, C.F.; Hamilton, P.B. Impairment of phagocytosis in chicken monocytes during aflatoxicosis. *Poult. Sci.* **1979**, *58*, 562–566.
163. Neldon-Ortiz, D.L.; Qureshi, M.A. Direct and microsomal activated aflatoxin B₁ exposure and its effects on turkey peritoneal macrophage functions *in vitro*. *Toxicol. Appl. Pharmacol.* **1991**, *109*, 432–442.
164. Neldon-Ortiz, D.L.; Qureshi, M.A. The effects of direct and microsomal activated aflatoxin B₁ on chicken peritoneal macrophages *in vitro*. *Vet. Immunol. Immunopathol.* **1992**, *31*, 61–76.
165. Chang, C.F.; Hamilton, P.B. Refractory phagocytosis by chicken thrombocytes during aflatoxicosis. *Poult. Sci.* **1979**, *58*, 559–561.
166. Ghosh, R.C.; Chauhan, H.V.; Roy, S. Immunosuppression in broilers under experimental aflatoxicosis. *Br. Vet. J.* **1990**, *146*, 457–462.

167. Giambrone, J.J.; Ewert, D.L.; Wyatt, R.D.; Eidson, C.S. Effect of aflatoxin on the humoral and cell-mediated immune systems of the chicken. *Am. J. Vet. Res.* **1978**, *39*, 305–308.
168. Chen, K.; Yuan, S.; Chen, J.; Peng, X.; Wang, F.; Cui, H.; Fang, J. Effects of sodium selenite on the decreased percentage of T cell subsets, contents of serum IL-2 and IFN- γ induced by aflatoxin B₁ in broilers. *Res. Vet. Sci.* **2013**, *95*, 143–145.
169. Chen, J.; Chen, K.; Yuan, S.; Peng, X.; Fang, J.; Wang, F.; Cui, H.; Chen, Z.; Yuan, J.; Geng, Y. Effects of aflatoxin B₁ on oxidative stress markers and apoptosis in spleens in broilers. *Toxicol. Ind. Health* **2013**, doi:10.1177/0748233713500819.
170. Wang, F.; Shu, G.; Peng, X.; Fang, J.; Chen, K.; Cui, H.; Chen, Z.; Zuo, Z.; Deng, J.; Gene, Y.; Lai, W. Protective effects of sodium selenite against aflatoxin-B₁ induced oxidative stress and apoptosis in broiler spleen. *Int. J. Environ. Res. Public Health* **2013**, *10*, 2834–2844.
171. He, Y.; Fang, J.; Peng, X.; Cui, H.; Zuo, Z.; Deng, J.; Chen, Z.; Lai, W.; Shu, G.; Tang, L. Effects of sodium selenite on aflatoxin B₁-induced decrease of ileac T cell and the mRNA contents of IL-2, IL-6, and TNF- α in broilers. *Biol. Trace Elem. Res.* **2014**, *159*, 167–173.
172. Jiang, M.; Peng, X.; Fang, J.; Cui, H.; Yu, Z.; Chen, Z. Effects of aflatoxin B₁ on T-cell subsets and mRNA expression of cytokines in the intestine of broilers. *Int. J. Mol. Sci.* **2015**, *16*, 6945–6959.
173. Campbell, M.L. Jr.; May, J.D.; Huff, W.E.; Doerr, J.A. Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis. *Poult. Sci.* **1983**, *62*, 2138–2144.
174. Pier, A.C.; Heddleston, K.L.; Boney, W.A.; Lukert, P.K. The effect of aflatoxin on immunity. *Proc. XIX Con. Mund. Med. Vet. Zootech.* **1971**, *1*, 216–219.
175. Stewart, R.G.; Skeeles, J.K.; Wyatt, R.D.; Brown, J.; Page, R.K.; Russell, I.D.; Lukert, P.D. The effect of aflatoxin on complement activity in broiler chickens. *Poult. Sci.* **1985**, *64*, 616–619.
176. Azzam, A.H.; Gabal, M.A. Interaction of aflatoxin in the feed and immunization against selected infectious diseases. I. Infectious bursal disease. *Avian Pathol.* **1997**, *26*, 317–325.
177. Gabal, M.A.; Azzam, A.H. Interaction of aflatoxin in the feed and immunization against selected infectious diseases in poultry. II. Effect on one-day-old layer chicks simultaneously vaccinated against Newcastle disease, infectious bronchitis and infectious bursal disease. *Avian Pathol.* **1998**, *27*, 290–295.
178. Boulton, S.L.; Dick, J.W.; Hughes, B.L. Effects of dietary aflatoxin and ammonia-inactivated aflatoxin on Newcastle disease antibody titers in layer-breeders. *Avian Dis.* **1982**, *26*, 1–6.
179. Hegazy, S.M.; Azzam, A.; Gabal, M.A. Interaction of naturally occurring aflatoxins in poultry feed and immunization against fowl cholera. *Poult. Sci.* **1991**, *70*, 2425–2428.
180. Pier, A.C.; Heddleston, K.L. The effect of aflatoxin on immunity in turkeys. I. Impairment of actively acquired resistance to bacterial challenge. *Avian Dis.* **1970**, *14*, 797–809.
181. Pier, A.C. Effects of aflatoxin on immunity. *J. Am. Vet. Med. Assoc.* **1973**, *163*, 1268–1269.
182. Boonchavit, B.; Hamilton, P.B. Interaction of aflatoxin and paratyphoid infection in broiler chickens. *Poult. Sci.* **1975**, *54*, 1567–1573.
183. Hegazy, S.M.; Adachi, Y. Comparison of the effects of dietary selenium, zinc, and selenium and zinc supplementation on growth and immune response between chick groups that were inoculated with *Salmonella* and aflatoxin or *Salmonella*. *Poult. Sci.* **2000**, *79*, 331–335.

184. Wyatt, R.D.; Hamilton, P.B. Interaction between aflatoxicosis and a natural infection of chickens with *Salmonella*. *Appl. Microbiol.* **1975**, *30*, 870–872.
185. Edds, G.T.; Nair, K.P.; Simpson, C.F. Effect of aflatoxin B₁ on resistance in poultry against cecal coccidiosis and Marek's disease. *Am. J. Vet. Res.* **1973**, *34*, 819–826.
186. Edds, G.T.; Simpson, C.F. Cecal coccidiosis in poultry as affected by prior exposure to aflatoxin B₁. *Am. J. Vet. Res.* **1976**, *37*, 65–68.
187. Witlock, D.R.; Wyatt, R.D.; Anderson, W.I. Relationship between *Eimeria adenoeides* infection and aflatoxicosis in turkey poults. *Poult. Sci.* **1982**, *61*, 1293–1297.
188. Wyatt, R.D.; Ruff, M.D.; Page, R.K. Interaction of aflatoxin with *Eimeria tenella* infection and monensin in young broiler chickens. *Avian Dis.* **1975**, *19*, 730–740.
189. Hamilton, P.B.; Harris, J.R. Interaction of aflatoxicosis with *Candida albicans* infections and other stresses in chickens. *Poult. Sci.* **1971**, *50*, 906–912.
190. Chang, C.F.; Hamilton, P.B. Increased severity and new symptoms of infectious bursal disease during aflatoxicosis in broiler chickens. *Poult. Sci.* **1982**, *61*, 1061–1068.
191. Giambone, J.J.; Partadiredja, M.; Eidson, C.S.; Kleven, S.H.; Wyatt, R.D. Interaction of aflatoxin with infectious bursal disease virus infection in young chickens. *Avian Dis.* **1978**, *22*, 431–439.
192. Batra, P.; Pruthi, A.K.; Sadana, J.R. Effect of aflatoxin B₁ on the efficacy of turkey herpesvirus vaccine against Marek's disease. *Res. Vet. Sci.* **1991**, *51*, 115–119.
193. Tessari, E.N.; Oliveira, C.A.; Cardoso, A.L.; Ledoux, D.R.; Rottinghaus, G.E. Effects of aflatoxin B₁ and fumonisin B₁ on body weight, antibody titres and histology of broiler chicks. *Br. Poult. Sci.* **2006**, *47*, 357–364.
194. Yunus, A.W.; Ghareeb, K.; Abd-El-Fattah, A.A.; Twaruzek, M.; Böhm, J. Gross intestinal adaptations in relation to broiler performance during chronic aflatoxin exposure. *Poult. Sci.* **2011**, *90*, 1683–1689.
195. Yunus, A.W.; Razzazi-Fazeli, E.; Bohm, J. Aflatoxin B₁ in affecting broiler's performance, immunity, and gastrointestinal tract: A review of history and contemporary issues. *Toxins* **2011**, *3*, 566–590.
196. Applegate, T.J.; Schatzmayr, G.; Prickel, K.; Troche, C.; Jiang, Z. Effect of aflatoxin culture on intestinal function and nutrient loss in laying hens. *Poult. Sci.* **2009**, *88*, 1235–1241.
197. Yang, J.; Bai, F.; Zhang, K.; Lv, X.; Bai, S.; Zhao, L.; Peng, X.; Ding, X.; Li, Y.; Zhang, J. Effects of feeding corn naturally contaminated with AFB₁ and AFB₂ on performance and aflatoxin residues in broilers. *Czech. J. Anim. Sci.* **2012**, *57*, 506–515.
198. Ledoux, D.R.; Rottinghaus, G.E.; Bermudez, A.J.; Alonso-Debolt, M. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. *Poult. Sci.* **1999**, *78*, 204–210.
199. Hamilton, P.B.; Garlich, J.D. Failure of vitamin supplementation to alter the fatty liver syndrome caused by aflatoxin. *Poult. Sci.* **1972**, *51*, 688–692.
200. Hamilton, P.B.; Tung, H.T.; Wyatt, R.D.; Donaldson, W.E. Interaction of dietary aflatoxin with some vitamin deficiencies. *Poult. Sci.* **1974**, *53*, 871–877.
201. Rogers, S.R.; Pesti, G.M.; Wyatt, R.D. Effect of tryptophan supplementation on aflatoxicosis in laying hens. *Poult. Sci.* **1991**, *70*, 307–312.

202. Hamilton, P.B.; Tung, H.T.; Harris, J.R.; Gainer, J.H.; Donaldson, W.E. The effect of dietary fat on aflatoxicosis in turkeys. *Poult. Sci.* **1972**, *51*, 165–170.
203. Richardson, K.E.; Nelson, L.A.; Hamilton, P.B. Effect of dietary fat level on dose response relationships during aflatoxicosis in young chickens. *Poult. Sci.* **1987**, *66*, 1470–1478.
204. Smith, J.W.; Hill, C.H.; Hamilton, P.B. The effect of dietary modifications on aflatoxicosis in the broiler chicken. *Poult. Sci.* **1971**, *50*, 768–774.
205. Kermanshahi, H.; Akbari, M.R.; Maleki, M.; Behgar, M. Effect of prolonged low level inclusion of aflatoxin B₁ into diet on performance, nutrient digestibility, histopathology and blood enzymes of broiler chickens. *J. Anim. Vet. Adv.* **2007**, *6*, 686–692.
206. Verma, J.; Swain, B.K.; Johri, T.S. Effect of various levels of aflatoxin and ochratoxin A and combinations thereof on protein and energy utilisation in broilers. *J. Sci. Food Agric.* **2002**, *82*, 1412–1417.
207. Verma, J.; Johri, T.S.; Swain, B.K. Effect of aflatoxin, ochratoxin and their combination on protein and energy utilisation in white leghorn laying hens. *J. Sci. Food Agric.* **2007**, *87*, 760–764.
208. Ruff, M.D.; Wyatt, R.D. Intestinal absorption of L-methionine and glucose in chickens with aflatoxicosis. *Toxicol. Appl. Pharmacol.* **1976**, *37*, 257–262.
209. Oliveira, C.A.; Kobashigawa, E.; Reis, T.A.; Mestieri, L.; Albuquerque, R.; Corrêa, B. Aflatoxin B₁ residues in eggs of laying hens fed a diet containing different levels of the mycotoxin. *Food Addit. Contam.* **2000**, *17*, 459–462.
210. Celik, I.; Oguz, H.; Demet, O.; Boydak, M.; Donmez, H.H.; Sur, E.; Nizamlioglu, F. Embryotoxicity assay of aflatoxin produced by *Aspergillus parasiticus* NRRL 2999. *Br. Poult. Sci.* **2000**, *41*, 401–409.
211. Dietert, R.R.; Qureshi, M.A.; Nanna, U.C.; Bloom, S.E. Embryonic exposure to aflatoxin-B₁: Mutagenicity and influence on development and immunity. *Environ. Mutagen.* **1985**, *7*, 715–725.
212. Edrington, T.S.; Harvey, R.B.; Kubena, L.F. Toxic effects of aflatoxin B₁ and ochratoxin A, alone and in combination, on chicken embryos. *Bull. Environ. Contam. Toxicol.* **1995**, *54*, 331–336.
213. Oznurlu, Y.; Celik, I.; Sur, E.; Ozaydin, T.; Oğuz, H.; Altunbaş, K. Determination of the effects of aflatoxin B₁ given *in ovo* on the proximal tibial growth plate of broiler chickens: Histological, histometric and immunohistochemical findings. *Avian Pathol.* **2012**, *41*, 469–477.
214. Sur, E.; Celik, I. Effects of aflatoxin B₁ on the development of the bursa of Fabricius and blood lymphocyte acid phosphatase of the chicken. *Br. Poult. Sci.* **2003**, *44*, 558–566.
215. Williams, J.G.; Deschl, U.; William, G.M. DNA damage in fetal liver cells of turkey and chicken eggs dosed with aflatoxin B₁. *Arch. Toxicol.* **2011**, *85*, 1167–1172.
216. Perrone, C.E.; Ahr, H.J.; Duan, J.D.; Jeffrey, A.M.; Schmidt, U.; Williams, G.M.; Enzmann, H.H. Embryonic turkey liver: Activities of biotransformation enzymes and activation of DNA-reactive carcinogens. *Arch. Toxicol.* **2004**, *78*, 589–598.
217. Sur, E.; Celik, I.; Oznurlu, Y.; Aydin, M.F.; Oguz, H.; Kurtoglu, V.; Ozaydin, T. Enzyme histochemical and serological investigations on the immune system from chickens treated *in ovo* with aflatoxin B₁ (AFB₁). *Revue Méd. Vét.* **2011**, *162*, 443–448.

218. Ul-Hassan, Z.; Khan, M.Z.; Khan, A.; Javed, I. Immunological status of the progeny of breeder hens kept on ochratoxin A (OTA)- and aflatoxin B₁ (AFB₁)-contaminated feeds. *J. Immunotoxicol.* **2012**, *9*, 381–391.
219. Lilleberg, S.L.; Cabonce, M.A.; Raju, N.R.; Wagner, L.M.; Kier, L.D. Alterations in the structural gene and the expression of *p53* in rat liver tumors induced by aflatoxin B₁. *Mol. Carcinog.* **1992**, *6*, 159–172.
220. Harris, A.J.; Shaddock, J.G.; Manjanatha, M.G.; Lisenbey, J.A.; Casciano, D.A. Identification of differentially expressed genes in aflatoxin B₁-treated cultured primary rat hepatocytes and Fischer 344 rats. *Carcinogenesis* **1998**, *19*, 1451–1458.
221. Merrick, B.A.; Phadke, D.P.; Auerbach, S.S.; Mav, D.; Stiegelmeier, S.M.; Shah, R.R.; Tice, R.R. RNA-Seq profiling reveals novel hepatic gene expression pattern in aflatoxin B₁ treated rats. *PLoS ONE* **2013**, *8*, e61768.
222. Dugyala, R.R.; Sharma, R.P. The effect of aflatoxin B₁ on cytokine mRNA and corresponding protein levels in peritoneal macrophages and splenic lymphocytes. *Int. J. Immunopharmacol.* **1996**, *18*, 599–608.
223. Han, S.H.; Jeon, Y.J.; Yea, S.S.; Yang, K.H. Suppression of the interleukin-2 gene expression by aflatoxin B₁ is mediated through the down-regulation of the NF-AT and AP-1 transcription factors. *Toxicol. Lett.* **1999**, *108*, 1–10.
224. Hinton, D.M.; Myers, M.J.; Raybourne, R.A.; Francke-Carroll, S.; Sotomayor, R.E.; Shaddock, J.; Warbritton, A.; Chou, M.W. Immunotoxicity of aflatoxin B₁ in rats: Effects on lymphocytes and the inflammatory response in a chronic intermittent dosing study. *Toxicol. Sci.* **2003**, *73*, 362–377.
225. Meissonnier, G.M.; Pinton, P.; Laffitte, J.; Cossalter, A.M.; Gong, Y.Y.; Wild, C.P.; Bertin, G.; Galtier, P.; Oswald, I.P. Immunotoxicity of aflatoxin B₁: Impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. *Toxicol. Appl. Pharmacol.* **2008**, *231*, 142–149.
226. Qian, G.; Tang, L.; Guo, X.; Wang, F.; Massey, M.E.; Su, J.; Guo, T.L.; Williams, J.H.; Phillips, T.D.; Wang, J.S. Aflatoxin B₁ modulates the expression of phenotypic markers and cytokines by splenic lymphocytes of male F344 rats. *J. Appl. Toxicol.* **2014**, *34*, 241–249.
227. Murarolli, R.A. Effects of Aflatoxin B₁ (AFB₁) on Hepatic Gene Expression in Pigs and Turkeys. Doctoral Dissertation. University of Missouri: Columbia, MO, USA, 2013.
228. Tilton, S.C.; Gerwick, L.G.; Hendricks, J.D.; Rosato, C.S.; Corley-Smith, G.; Givan, S.A.; Bailey, G.S.; Bayne, C.J.; Williams, D.E. Use of a rainbow trout oligonucleotide microarray to determine transcriptional patterns in aflatoxin B₁-induced hepatocellular carcinoma compared to adjacent liver. *Toxicol. Sci.* **2005**, *88*, 319–330.
229. Afanassieff, M.; Goto, R.M.; Ha, J.; Sherman, M.A.; Zhong, L.; Auffray, C.; Coudert, F.; Zoorob, R.; Miller, M.M. At least one class I gene in restriction fragment pattern-Y (*Rfp.-Y*), the second MHC gene cluster in the chicken, is transcribed, polymorphic, and shows divergent specialization in antigen binding region. *J. Immunol.* **2001**, *166*, 3324–3333.
230. Bauer, M.M.; Reed, K.M. Extended sequence of the turkey MHC *B*-locus and sequence variation in the highly polymorphic B-G loci. *Immunogenetics* **2011**, *63*, 209–221.

231. Chaves, L.D.; Krueth, S.B.; Bauer, M.M.; Reed, K.M. Sequence of a turkey BAC clone identifies MHC class III orthologs and supports ancient origins of immunological gene clusters. *Cytogenet. Genome Res.* **2011**, *132*, 55–63.
232. Chaves, L.D.; Krueth, S.B.; Reed, K.M. Characterization of the turkey MHC chromosome through genetic and physical mapping. *Cytogenet. Genome Res.* **2007**, *117*, 213–220.
233. Chaves, L.D.; Krueth, S.B.; Reed, K.M. Defining the turkey MHC: Sequence and genes of the B locus. *J. Immunol.* **2009**, *183*, 6530–6537.
234. Delany, M.E.; Robinson, C.M.; Goto, R.M.; Miller, M.M. Architecture and organization of chicken microchromosome 16: Order of the *NOR*, *MHC-Y*, and *MHC-B* subregions. *J. Hered.* **2009**, *100*, 507–514.
235. Kaufman, J.; Milne, S.; Göbel, T.W.F.; Walker, B.A.; Jacob, J.P.; Auffray, C.; Zoorob, R.; Beck, S. The chicken B locus is a minimal essential major histocompatibility complex. *Nature* **1999**, *401*, 923–925.
236. Miller, M.M.; Goto, R.; Bernot, A.; Zoorob, R.; Auffray, C.; Bumstead, N.; Briles, W.E. Two *Mhc* class I and two *Mhc* class II genes map to the chicken *Rfp.-Y*. system outside the *B* complex. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4397–4401.
237. Reed, K.M.; Bauer, M.M.; Monson, M.S.; Benoit, B.; Chaves, L.D.; O’Hare, T.H.; Delany, M.E. Defining the turkey MHC: Identification of expressed class I- and class IIB-like genes independent of the *MHC-B*. *Immunogenetics* **2011**, *63*, 753–771.
238. Monson, M.S.; Settlege, R.E.; Mendoza, K.M.; Rawal, S.; El-Nezami, H.S.; Coulombe, R.A.; Reed, K.M. Modulation of the spleen transcriptome in domestic turkey (*Meleagris gallopavo*) in response to aflatoxin B₁ and probiotics. *Immunogenetics* **2015**, *67*, 163–178.
239. Rustemeyer, S.M.; Lamberson, W.R.; Ledoux, D.R.; Wells, K.; Austin, K.J.; Cammack, K.M. Effects of dietary aflatoxin on the hepatic expression of apoptosis genes in growing barrows. *J. Anim. Sci.* **2005**, *89*, 916–925.
240. Monson, M.S.; Settlege, R.E.; McMahan, K.W.; Mendoza, K.M.; Rawal, S.; el-Nezami, H.S.; Coulombe, R.A.; Reed, K.M. Response of the hepatic transcriptome to aflatoxin B₁ in domestic turkey (*Meleagris gallopavo*). *PLoS ONE* **2014**, *9*, e100930.
241. Bothast, R.J.; Nofsinger, G.W.; Lagoda, A.A.; Black, L.T. Integrated process for ammonia inactivation of aflatoxin-contaminated corn and ethanol fermentation. *Appl. Environ. Microbiol.* **1982**, *43*, 961–963.
242. Brekke, O.L.; Sinnhuber, R.O.; Peplinski, A.J.; Wales, J.H.; Putnam, G.B.; Lee, D.J.; Ciegler, A. Aflatoxin in corn: Ammonia inactivation and bioassay with rainbow trout. *Appl. Environ. Microbiol.* **1977**, *34*, 34–37.
243. Jalili, M.; Jinap, S.; Son, R. The effect of chemical treatment on reduction of aflatoxins and ochratoxin a in black and white pepper during washing. *Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess.* **2011**, *28*, 485–493.
244. Mann, G.E.; Gardner, H.K.; Booth, A.N.; Gumbmann, M.R. Aflatoxin inactivation. Chemical and biological properties of ammonia and methylamine treated cottonseed meal. *J. Agric. Food Chem.* **1971**, *19*, 1155–1158.

245. Vesonder, R.F.; Beckwith, A.C.; Ciegler, A.; Dimler, R.J. Ammonium hydroxide treatment of aflatoxin B₁. Some chemical characteristics and biological effects. *J. Agric. Food Chem.* **1975**, *23*, 242–243.
246. Weng, C.Y.; Martinez, A.J.; Park, D.L. Efficacy and permanency of ammonia treatment in reducing aflatoxin levels in corn. *Food Addit. Contam.* **1994**, *11*, 649–658.
247. Codifer, L.P., Jr.; Mann, G.E.; Dollear, F.G. Aflatoxin inactivation: Treatment of peanut meal with formaldehyde and calcium hydroxide. *J. Am. Oil Chem. Soc.* **1976**, *53*, 204–206.
248. Patel, U.D.; Govindarajan, P.; Dave, P.J. Inactivation of aflatoxin B₁ by using the synergistic effect of hydrogen peroxide and gamma radiation. *Appl. Environ. Microbiol.* **1989**, *55*, 465–467.
249. Moerck, K.E.; McElfresh, P.; Wohlman, A.; Hilton, B.W. Aflatoxin destruction in corn using sodium bisulfite, sodium hydroxide and aqueous ammonia. *J. Food Prot.* **1980**, *43*, 571–574.
250. Yang, C.Y. Comparative studies on the detoxification of aflatoxins by sodium hypochlorite and commercial bleaches. *Appl. Microbiol.* **1972**, *24*, 885–890.
251. Gregory, J.F., 3rd; Edds, G.T. Effect of dietary selenium on the metabolism of aflatoxin B₁ in turkeys. *Food Chem. Toxicol.* **1984**, *22*, 637–642.
252. Diaz, G.J.; Cortés, A.; Botero, L. Evaluation of the ability of a feed additive to ameliorate the adverse effects of aflatoxins in turkey poults. *Br. Poult. Sci.* **2009**, *50*, 240–250.
253. Kubena, L.F.; Harvey, R.B.; Huff, W.E.; Corrier, D.E.; Phillips, T.D.; Rottinghaus, G.E. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. *Poult. Sci.* **1990**, *69*, 1078–1086.
254. Kubena, L.F.; Harvey, R.B.; Phillips, T.D.; Corrier, D.E.; Huff, W.E. Diminution of aflatoxicosis in growing chickens by the dietary addition of a hydrated, sodium calcium aluminosilicate. *Poult. Sci.* **1990**, *69*, 727–735.
255. Kubena, L.F.; Huff, W.E.; Harvey, R.B.; Yersin, A.G.; Elissalde, M.H.; Witzel, D.A.; Giroir, L.E.; Phillips, T.D.; Petersen, H.D. Effects of a hydrated sodium calcium aluminosilicate on growing turkey poults during aflatoxicosis. *Poult. Sci.* **1991**, *70*, 1823–1830.
256. Kubena, L.F.; Harvey, R.B.; Phillips, T.D.; Clement, B.A. Effect of hydrated sodium calcium aluminosilicates on aflatoxicosis in broiler chicks. *Poult. Sci.* **1993**, *72*, 651–657.
257. Zhao, J.; Shirley, R.B.; Dibner, J.D.; Uraizee, F.; Officer, M.; Kitchell, M.; Vazquez-Anon, M.; Knight, C.D. Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks. *Poult. Sci.* **2010**, *89*, 2147–2156.
258. 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. Effect of low levels of aflatoxin B₁ on performance, biochemical parameters, and aflatoxin B₁ in broiler liver tissues in the presence of monensin and sodium bentonite. *Poult. Sci.* **2011**, *90*, 48–58.
259. Coulombe, R.A.; Guarisco, J.A.; Klein, P.J.; Hall, J.O. Chemoprevention of aflatoxicosis in poultry by dietary butylated hydroxytoluene. *Anim. Feed Sci. Technol.* **2005**, *121*, 217–225.
260. Guarisco, J.A.; Hall, J.O.; Coulombe, R.A., Jr. Mechanisms of butylated hydroxytoluene chemoprevention of aflatoxicosis-inhibition of aflatoxin B₁ metabolism. *Toxicol. Appl. Pharmacol.* **2008**, *227*, 339–346.

261. Guarisco, J.A.; Hall, J.O.; Coulombe, R.A., Jr. Butylated hydroxytoluene chemoprevention of aflatoxicosis—Effects on aflatoxin B₁ bioavailability, hepatic DNA adduct formation, and biliary excretion. *Food Chem. Toxicol.* **2008**, *46*, 3727–3731.
262. Klein, P.J.; van Vleet, T.R.; Hall, J.O.; Coulombe, R.A., Jr. Effects of dietary butylated hydroxytoluene on aflatoxin B₁-relevant metabolic enzymes in turkeys. *Food Chem. Toxicol.* **2003**, *41*, 671–678.
263. Larsen, C.; Ehrich, M.; Driscoll, C.; Gross, W.B. Aflatoxin-antioxidant effects on growth of young chicks. *Poult. Sci.* **1985**, *64*, 2287–2291.
264. Gowda, N.K.; Ledoux, D.R.; Rottinghaus, G.E.; Bermudez, A.J.; Chen, Y.C. Antioxidant efficacy of curcuminoids from turmeric (*Curcuma longa* L.) powder in broiler chickens fed diets containing aflatoxin B₁. *Br. J. Nutr.* **2009**, *102*, 1629–1634.
265. Bagherzadeh Kasmani, F.; Karimi Torshizi, M.A.; Allameh, A.; Shariatmadari, F. A novel aflatoxin-binding *Bacillus* probiotic: Performance, serum biochemistry, and immunological parameters in Japanese quail. *Poult. Sci.* **2012**, *91*, 1846–1853.
266. Oatley, J.T.; Rarick, M.D.; Ji, G.E.; Linz, J.E. Binding of aflatoxin B₁ to *Bifidobacteria* *in vitro*. *J. Food Prot.* **2000**, *63*, 1133–1136.
267. Peltonen, K.; El-Nezami, H.; Haskard, C.; Ahokas, J.; Salminen, S. Aflatoxin B₁ binding by dairy strains of lactic acid bacteria and *Bifidobacteria*. *J. Dairy Sci.* **2001**, *84*, 2152–2156.
268. Shahin, A.A.M. Removal of aflatoxin B₁ from contaminated liquid media by dairy lactic acid bacteria. *Int. J. Agric. Biol.* **2007**, *9*, 71–75.
269. Topcu, A.; Bulat, T.; Wishah, R.; Boyac, I.H. Detoxification of aflatoxin B₁ and patulin by *Enterococcus faecium* strains. *Int. J. Food Microbiol.* **2010**, *139*, 202–205.
270. Deabes, M.M.; Darwish, H.R.; Abdel-Aziz, K.B.; Farag, I.M.; Nada, S.A.; Tawfek, N.S. Protective effects of *Lactobacillus rhamnosus* GG on aflatoxins-induced toxicities in male albino mice. *J. Environ. Anal. Toxicol.* **2012**, *2*, 132.
271. El-Nezami, H.; Salminen, S.J.; Ahokas, J. Biological control of food carcinogens with use of *Lactobacillus* GG. *Nutr. Today* **1996**, *21*, 41S–42S.
272. El-Nezami, H.; Kankaanpää, P.; Salminen, S.; Ahokas, J. Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B₁. *Food Chem. Toxicol.* **1998**, *36*, 321–326.
273. El-Nezami, H.; Kankaanpää, P.; Salminen, S.; Ahokas, J. Physicochemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. *J. Food Prot.* **1998**, *61*, 466–468.
274. El-Nezami, H.; Mykkänen, H.; Kankaanpää, P.; Salminen, S.; Ahokas, J. Ability of *Lactobacillus* and *Propionibacterium* strains to remove aflatoxin B₁ from the chicken duodenum. *J. Food Prot.* **2000**, *63*, 549–552.
275. Fazell, M.R.; Hajimohammadali, M.; Moshkani, A.; Samadi, N.; Jamalifar, H.; Khoshayand, M.R.; Vaghari, E.; Pouragahi, S. Aflatoxin B₁ binding capacity of autochthonous strains of lactic acid bacteria. *J. Food Prot.* **2009**, *72*, 189–192.
276. Gratz, S.; Mykkänen, H.; Ouwehand, A.C.; Juvonen, R.; Salminen, S.; el-Nezami, H. Intestinal mucus alters the ability of probiotic bacteria to bind aflatoxin B₁ *in vitro*. *Appl. Environ. Microbiol.* **2004**, *70*, 6306–6308.

277. Gratz, S.; Täubel, M.; Juvonen, R.O.; Viluksela, M.; Turner, P.C.; Mykkänen, H.; el-Nezami, H. *Lactobacillus rhamnosus* strain GG modulates intestinal absorption, fecal excretion and toxicity of aflatoxin B₁ in rats. *Appl. Environ. Microbiol.* **2006**, *72*, 7398–7400.
278. Gratz, S.; Wu, Q.K.; el-Nezami, H.; Juvonen, R.O.; Mykkänen, H.; Turner, P.C. *Lactobacillus rhamnosus* strain GG reduces aflatoxin B₁ transport, metabolism, and toxicity in Caco-2 cells. *Appl. Environ. Microbiol.* **2007**, *73*, 3958–3964.
279. Haskard, C.; Binnion, C.; Ahokas, J. Factors affecting the sequestration of aflatoxin by *Lactobacillus rhamnosus* strain GG. *Chem. Biol. Interact.* **2000**, *128*, 39–49.
280. Haskard, C.A.; el-Nezami, H.S.; Kankaanpää, P.E.; Salminen, S.; Ahokas, J.T. Surface binding of aflatoxin B₁ by lactic acid bacteria. *Appl. Environ. Microbiol.* **2001**, *67*, 3086–3091.
281. Hernandez-Mendoza, A.; Garcia, H.S.; Steele, J.L. Screening of *Lactobacillus casei* strains for their ability to bind aflatoxin B₁. *Food Chem. Toxicol.* **2009**, *47*, 1064–1068.
282. Hernandez-Mendoza, A.; Guzman de Peña, D.; Garcia, H.S. Key role of teichoic acids on aflatoxin B₁ binding by probiotic bacteria. *J. Appl. Microbiol.* **2009**, *107*, 395–403.
283. Hernandez-Mendoza, A.; Rivas-Jimenez, L.; Garcia, H.S. Assessment of aflatoxin B₁ binding to *Lactobacillus reuteri* by microscopy and fluorescence techniques. *Food Biotechnol.* **2011**, *25*, 140–150.
284. Kankaanpää, P.; Tuomola, E.; El-Nezami, H.; Ahokas, J.; Salminen, S.J. Binding of aflatoxin B₁ alters the adhesion properties of *Lactobacillus rhamnosus* strain GG in a Caco-2 model. *J. Food Prot.* **2000**, *63*, 412–414.
285. Lahtinen, S.J.; Haskard, C.A.; Ouwehand, A.C.; Salminen, S.J.; Ahokas, J.T. Binding of aflatoxin B₁ to cell wall components of *Lactobacillus rhamnosus* strain GG. *Food Addit. Contam.* **2004**, *21*, 158–164.
286. Lee, Y.K.; el-Nezami, H.; Haskard, C.A.; Gratz, S.; Puong, K.Y.; Salminen, S.; Mykkänen, H. Kinetics of adsorption and desorption of aflatoxin B₁ by viable and nonviable bacteria. *J. Food Prot.* **2003**, *66*, 426–430.
287. Peltonen, K.D.; el-Nezami, H.S.; Salminen, S.J.; Ahokas, J.T. Binding of aflatoxin B₁ by probiotic bacteria. *J. Sci. Food Agric.* **2000**, *80*, 1942–1945.
288. Turbic, A.; Ahokas, J.T.; Haskard, C.A. Selective *in vitro* binding of dietary mutagens, individually or in combination, by lactic acid bacteria. *Food Addit. Contam.* **2002**, *19*, 144–152.
289. El-Nezami, H.; Mykkänen, H.; Kankaanpää, P.; Suomalaine, T.; Salminen, S.; Ahokas, J. Ability of a mixture of *Lactobacillus* and *Propionibacterium* to influence the faecal aflatoxin content in healthy Egyptian volunteers: A pilot clinical study. *Biosci. Microflora* **2000**, *19*, 41–45.
290. El-Nezami, H.S.; Polychronaki, N.N.; Ma, J.; Zhu, H.; Ling, W.; Salminen, E.K.; Juvonen, R.O.; Salminen, S.J.; Poussa, T.; Mykkänen, H.M. Probiotic supplementation reduces a biomarker for increased risk of liver cancer in young men from Southern China. *Am. J. Clin. Nutr.* **2006**, *83*, 1199–1203.
291. Lanza, G.M.; Washburn, K.W.; Wyatt, R.D.; Marks, H.L. Genetic variation of physiological response to aflatoxin in *Gallus domesticus*. *Theor. Appl. Genet.* **1982**, *63*, 207–212.
292. Lanza, G.M.; Washburn, K.W.; Wyatt, R.D.; Marks, H.L. Effect of dietary aflatoxin concentration on the assessment of genetic variability of response in a randombred population of chickens. *Genetics* **1983**, *104*, 123–131.

293. Manning, R.O.; Wyatt, R.D.; Marks, H.L.; Fletcher, O.J. Effects of dietary aflatoxin in aflatoxin-resistant and control lines of chickens. *Poult. Sci.* **1990**, *69*, 922–928.
294. Scott, T.R.; Rowland, S.M.; Rodgers, R.S.; Bodine, A.B. Genetic selection for aflatoxin B₁ resistance influences chicken T-cell and thymocyte proliferation. *Dev. Comp. Immunol.* **1991**, *15*, 383–391.
295. Wyatt, R.D.; Marks, H.L.; Manning, R.O. Selection for resistance to aflatoxin in chickens. *Poult. Sci.* **1987**, *66*, 1901–1904.
296. Marks, H.L.; Wyatt, R.D. Genetic resistance to aflatoxin in Japanese quail. *Science* **1979**, *206*, 1329–1330.
297. Pegram, R.A.; Wyatt, R.D.; Marks, H.L. Comparative responses of genetically resistant and nonselected Japanese quail to dietary aflatoxin. *Poult. Sci.* **1985**, *64*, 266–272.
298. Pegram, R.A.; Wyatt, R.D.; Marks, H.L. The relationship of certain blood parameters to aflatoxin resistance in Japanese quail. *Poult. Sci.* **1986**, *65*, 1652–1658.
299. Rodeheaver, D.P.; Wyatt, R.D.; Marks, H.L. Relation of serum alpha-amylase to aflatoxin resistance in Japanese quail. *Avian Dis.* **1986**, *30*, 568–573.
300. Monson, M.S. Hepatotoxic and Immunomodulatory Transcriptome Responses to Aflatoxin B₁ in the Turkey (*Meleagris Gallopavo*). Ph.D. Thesis, University of Minnesota, Saint Paul, MN, USA, May 2015.
301. Coulombe, R.A. *USDA project: Functional Genomics to Enhance Aflatoxin Resistance in Poultry*. Utah State University: Logan, UT, USA, 2015.

© 2015 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).