Review Article

The Mechanism Underlying the Extreme Sensitivity of Duck to Aflatoxin B$_1$

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Received 7 March 2021; Revised 1 April 2021; Accepted 18 April 2021; Published 17 May 2021

Academic Editor: Daoud Ali

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Most metabolites of aflatoxin B$_1$ (AFB$_1$), especially exo-AFB$_1$-8,9-epoxide (AFBO), can induce the production of reactive oxygen species (ROS) to vary degrees, causing oxidative stress and liver damage, and ultimately induce liver cancer in humans and animals. Duck is one of the most sensitive animals to AFB$_1$, and severe economic losses are caused by duck AFB$_1$ poisoning every year, but the exact mechanism of this high sensitivity is still unclear. This review highlights significant advances in our understanding of the AFB$_1$ metabolic activation, like cytochrome P450s (CYPs), and AFB$_1$ metabolic detoxification, like glutathione S-transferases (GSTs) in poultry. In addition, AFB$_1$ may have other metabolic pathways in poultry, such as the mutual conversion of AFB$_1$ and aflatoxicol (AFL) and the process of AFBO to produce AFB$_1$-8,9-dihydrodiol (AFB$_1$-dhd) and further metabolize it into detoxification substances. This review also summarized some exogenous regulatory substances that can alleviate AFB$_1$-induced oxidative stress.

1. Introduction

Aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*, including aflatoxin B$_1$ (AFB$_1$), aflatoxin B$_2$, aflatoxin G$_1$, and aflatoxin G$_2$. AFB$_1$ is extremely toxic and carcinogenic, as classified by the International Agency for Research on Cancer (IARC), as a class 1A carcinogen [1]. AFB$_1$ generates a large amount of ROS during metabolism, which can cause oxidative damage [2] and reduce the level of antioxidant enzymes in animals [3] that eventually cause AFB$_1$’s toxic effects such as cell damage, DNA damage, protein damage, and lipid peroxidation [4]. AFB$_1$ has the strong liver toxicity and can reduce production performance, feed utilization [3], egg production, and immunosuppression in poultry [5]. Young ducks and turkeys are extremely susceptible to AFB$_1$ [5]. 3 µg/(kg·BW) of AFB$_1$ can cause significant damage to the DNA of duckling liver cells (Wang et al., 2009). 10–40 µg/(kg·BW) of AFB$_1$ can cause significant changes in the expression of many genes related to the oxidation-reduction process, metabolic toxins and detoxification process, and carcinogenesis in duck [6]. However, chickens (500 µg/kg AFB$_1$) and rats (100–1,000 µg/kg AFB$_1$) are relatively insensitive [5]. In China’s national feed hygiene standard “GB 13078-2017,” the concentration limit of AFB$_1$ in feed for meat ducks and laying ducks is ≤15 µg/kg, and the concentration limit of AFB$_1$ in feed for ducklings is ≤10 µg/kg.

Duck is one of the most important poultry for humans. Asia is the most important region for duck meat production (the ratio is 82.2%), followed by Europe with 12.4% [7]. In 2016, there were 1.24 billion ducks in the world and 1.1 billion (the ratio is 89.0%) in Asia. China raises more than 70% of the world’s ducks, the annual production of meat ducks is maintained at more than 2 billion, and the stock of laying ducks is about 250 million. The consumption of duck eggs accounts for 10–30% of the total consumption of eggs in China and Southeast Asia. Notably, most areas of China and Southeast Asian countries are located in tropical and temperate zones, and the warm and humid summers cause widespread moldy feed. AFB$_1$ widely contaminates grain and
animal feed worldwide [8, 9]. Of feed ingredients and compound feeds, 1,569 in different China provinces were checked in 2016–2017. The pollution rate of AFB1, is as high as 83.3% [10]. As the pollution of AFB1 in feed is difficult to avoid, ducks have biological defects susceptible to AFB1, causing countless economic losses due to duck AFB1 poisoning each year. This review summarizes and discusses the possible mechanisms of ducks’ high sensitivity to AFB1, and the feasibility of regulating ducks’ AFB1 metabolism through exogenous substances, thereby enhancing ducks’ tolerance to AFB1. This article is aimed at providing a theoretical basis for the research of exogenous substances to prevent and regulate duck aflatoxin poisoning.

2. Metabolic Process of AFB1

After AFB1 is absorbed in the intestine, it is converted into various metabolites by many cytochrome oxidase P450 family members (CYP 450 s, CYPs) in the liver. Aflatoxins M1 (AFM1) and aflatoxin P1 (AFP1) can be excreted directly through urine or milk [11]. The unabsorbed AFB1 and part of its metabolites are excreted through feces. As the liver’s dominant enzyme to metabolize AFB1, CYP1A2 can metabolize AFB1 into AFM1 and aflatoxin Q1 (AFQ1). CYP3A4 can metabolize AFB1 into AFQ1 and exo-AFB1-8,9-epoxide (AFBO). Primates mainly produce AFQ1 and AFP1, while poultry mainly produces aflatoxicol (AFL) [12]. CYP450 participates in AFB1 activation into AFBO; so, it is closely related to the occurrence of liver cancer. AFBO has oxidative activity and can induce excessive ROS formation, leading to DNA bases’ damage and inducing lipid peroxidation [13]. AFBO and ROS are then combined and metabolized and detoxified by some antioxidant enzymes and substances, such as GSTs, SOD, and GSH. AFBO can (1) combine with glutathione (GSH) under the activity of the body’s phase II metabolic enzymes, such as glutathione S-transferases (GSTs), to form AFB1-mercaptutic acid (AFB1-NAC) and be excreted through urine [14]; (2) combined with glucuronic acid and excreted through feces [15]; (3) combined with DNA to form AFB1-N’-guanine adduct and undergo the DNA repair process to quickly remove most of the AFB1-N’-GUA adducts and excrete them through urine; and (4) after binding to serum albumin, AFB1 mainly remains in the blood by the form of AFB1-lysine adduct [11]. The transformation process in animals is summarized in Figure 1.

The order of the sensitivity of different poultries to AFB1 from high to low is ducklings > turkey > goose > quail > chicks [16]. The 400 μg/kg AFB1, in the diet seriously affected turkeys’ growth performance and relative liver index. Chickens were not affected by this concentration, and their body weight increased by 3.3% [17]. Similarly, 220–400 μg/kg of AFB1 significantly reduced ducks’ performance but had a weak effect on chickens [18]. What causes different sensitivity of poultry to AFB1? A study of perfusion of isolated organs proved that poultry’s sensitivity to AFB1 is related to cell specificity (different species) but not to extracellular factors, such as the digestion and absorption process [19]. After AFB1 enters the liver, it undergoes two processes: metabolic activation and metabolic detoxification. AFB1 is metabolically activated by phase I metabolic enzymes, such as CYPs, to produce highly toxic AFBO and other metabolites. AFBO can be metabolized by phase II metabolic enzymes, such as GSTs, into low-toxic conjugates, and excreted from the body [11]. When the amount of AFBO produced exceeds the detoxification ability of phase II metabolic enzymes, AFBO will react or combine with DNA and proteins, resulting in cytotoxicity and genetic toxicity. Therefore, ducks’ high sensitivity to AFB1 may be related to excessive activation into harmful substances or insufficient detoxification of the harmful metabolites.

3. Metabolic Activation and Detoxification of AFB1 in Poultry

3.1. Metabolic Activation of AFB1 in Poultry. AFBO can cause oxidative stress, and AFBO is produced after AFB1 is activated by phase I enzyme metabolism. Therefore, reducing phase I metabolic enzyme activity can reduce the AFBO produced by metabolism and alleviate oxidative stress and other toxicities. Western countries raise and consume a large number of turkeys, while ducks are relatively few. Therefore, western countries have carried out a lot of research work on the metabolic mechanism of AFB1 in turkeys, but there are few studies on ducks. Turkeys are susceptible to AFB1 because their phase I enzymes can efficiently activate AFB1 [20]. An earlier study found that duck liver microsomes only need 50 seconds to metabolize AFB1 with LD50 concentration, while chickens need 32–100 minutes [21].

Interestingly, the content and activity of CYPs in the liver decrease with age in poultry. Young turkeys (9 d) activate AFB1 to AFBO more effectively than old turkeys (65 d) [22]. The same situation occurs in Ross broilers [23] and AA broilers [24]. The content and activity of liver CYPs on the first day after hatching were more than twice that of other ages, especially CYP1A1, CYP3A3, CYP2C, CYP2D, and CYP2H. It dropped rapidly to 28 days in the first week after hatching and then rose slightly to 56 days [23]. These studies indicate that the increase in age will affect phase I metabolic enzyme activity, affecting the toxicity and metabolic activation of AFB1 in animals.

Different CYPs metabolize AFB1 at different concentrations. CYP1A2 metabolizes human hepatocytes at lower concentrations (0.133 μM AFB1), and at higher concentrations (256 μM AFB1), they are metabolized by CYP3A4 [25]. Some enzymes are orthologous to human CYP1A2 and CYP3A4 in the turkey liver, CYP1A5 [26], and CYP3A37, respectively [27]. CYP1A5 metabolizes AFB1 into AFM1 and AFBO, and CYP3A37 metabolizes AFB1 into AFQ1 and AFBO. When 0.1–20 μM AFB1 is added to turkeys’ liver microsomes, it is mainly metabolized by CYP1A5 to produce AFBO. When 50 μM AFB1 is added, the proportion of AFBO produced by CYP1A5 and CYP3A37 is nearly equal, and 100–1,000 μM AFB1 is metabolized by CYP3A37 into AFBO [28]. Because the concentration of environmentally relevant AFB1 is mostly low-dose levels, CYP1A5 is an important enzyme involved in biological activation in turkeys and can regulate the production of AFBO. After ducklings were exposed to AFB1, the expression of CYP46A1, CYP3A9, and...
CYP4B1 in the liver was upregulated, while the expression of CYP2H1, CYP1A5, CYP27A1, CYP2K1, and CYP2F3 was downregulated [6].

Studies have found that the activation of AFB1, in turkeys [29] and broiler chickens [30] is mainly due to the role of CYP2A6 orthologues. CYP1A1 orthologues also participate in the formation of AFBO to a certain extent, but the effect of CYP3A4 on the formation of AFBO can be ignored. In the duck liver, CYP2A6 and CYP3A4 orthologues are the most important CYPs responsible for the activation of AFB1, and CYP1A1/2 orthologues are also involved in the formation of AFBO to a certain extent. The four CYP enzymes have differences in the activation of AFB1 and their enzyme activities, which may be one reason for the differences in poultry sensitivity to AFB1 [31]. In addition, it has been reported that fumonisin B1 (FB1) exposure significantly increased the activity of CYP1A1/2 and CYP3A4 orthologues in the duck liver, so that FB1 may cause more biological activation of AFB1 [32]. FB1 and AFB1 are often cocontaminated in feed; so, the combined toxicity to ducks may increase [33]. Although the difference in CYPs (type and activity) can partially explain turkeys' high sensitivity to AFB1, it is inconsistent with the apparent sensitivity of chickens, ducks, quail, and turkeys. Thus, it is not sufficient to fully explain the high sensitivity of ducks [34]. Therefore, it is necessary to continue investigating the biochemical and molecular biological

![Diagram of metabolic process of aflatoxin B1 (AFB1) in the animal liver.](image)

**Figure 1:** Metabolic process of aflatoxin B1 (AFB1) in the animal liver. (a) Aspergillus flavus grows in moldy feed, producing toxic metabolites, like AFB1. Poultry consume feed contaminated with AFB1. (b) In the liver, AFB1 produces exo-AFB1-8,9-epoxide (AFBO) under the action of phase I metabolic activating enzymes, such as cytochrome oxidase P450 (CYP 450), and binds to DNA and protein, causing gene damage and cytotoxicity and is potently carcinogenic. (c) At the same time, some phase I metabolic activating enzymes also convert AFB1 into low-toxic metabolites; or under the action of phase II metabolic detoxification enzymes, such as glutathione S-transferases (GSTs), AFB1 combines with antioxidants into nontoxic conjugates and is then excreted from the body to alleviate the toxicity of AFB1.
mechanisms of duck and turkey’s high sensitivity to AFB1, which is of great significance to the development of technologies to prevent and control the hazards of AFB1 [5].

3.2. Metabolic Detoxification of AFB1 in Poultry. Enhancing the activity of phase II enzymes can remove harmful AFBO and ROS faster, thereby alleviating oxidative stress and other toxicities. The high sensitivity of ducks to AFB1 may also be related to the lack of effective phase II detoxification enzymes, especially GSTs. The expression level of α-GST in the mouse liver is very high; so, it has a high tolerance to AFB1 [35]. Among the five GSTs in rats, α-GST and μ-GST can bind AFBO very effectively [36]. Poultry GSTs received less attention in the early stage [37] and gradually began to receive attention after 2000. At present, some GSTs found in poultry have been reported [11]. Compared with wild turkeys, commercial turkeys lack functional GST that has a sufficient affinity for AFBO, especially GSTA3 [38]. The process of domestication may lead to the loss of important detoxification GSTs genes, silence, or downregulation of expression, which may be an important reason for the sensitivity of commercial turkeys [39]. Interestingly, transcriptomic results showed that other organs and tissues, such as the cecal tonsil [40] and spleen [41], also helped enhance the tolerance of wild turkeys to AFB1. The modern poultry industry continuously selects and improves varieties, intensively breeds poultry, and shortens the breeding time. Since 1980, the sensitivity of poultry to AFB1 and external adverse factors has increased. This phenomenon suggests that we should reduce the adverse effects of mycotoxins and external diseases on poultry, while cultivating poultry with high feed conversion efficiency.

The activities of phase II metabolic enzymes, such as GSTs and AFB1 aldehyde reductase (AFAR), increase with the age of poultry, such as GSTA3, GSTA4, and EPHX1 in the liver of broilers [24]. Turkeys of all ages have low GST activity [22]. The lack of effective phase II enzyme detoxification may be another important reason why turkeys are susceptible to AFB1 [20]. AFB1 treatment increased the protein expression levels of GST Yc and Yc2 in turkeys’ liver [22]. A study amplified and identified the tGST genes (tGSTA1.1, tGSTA1.2, tGSTA1.3, tGSTA2, tGSTA3, and tGSTA4) in the turkey liver [42]. The expression of GST1, GST3, and GSTK1 genes in the liver of ducklings increased after exposure to AFB1 [6]. Recombined tGSTA can detoxify AFBO, but its liver form cannot, indicating that it can affect the affinity of tGSTA and AFBO by adjusting the modification of tGSTA [43]. It shows that it is feasible to modify and adjust the activity of GSTs by exogenous substances, thereby enhancing the tolerance of poultry to AFB1. Therefore, the low activity of metabolic detoxification enzymes, like GSTs, may be a critical factor in ducks’ high sensitivity to AFB1. GSTs play an essential role in the metabolic detoxification of AFB1. However, current research reports mostly identify the types of GSTs in poultry [42] or analyze GSTs that are significantly changed after AFB1 treatment [6]. There is a lack of further research on the specific detoxification effect of a certain GST in the process of duck metabolism AFB1.

3.3. Mutual Transformation of AFL and AFB1. Lozano and Diaz (2006) found that turkey and duck produced AFB1-GSH levels 3.25 times and 1.54 times that of chickens, respectively. This shows that the detoxification ability of GSTs in turkeys and ducks is not lower than that of chickens, and the differences in the types and activities of CYPs and GSTs cannot completely explain the reasons for the high sensitivity of ducks to AFB1. Therefore, there may be other toxicological mechanisms. AFL is one of the metabolites of AFB1, which can be converted to AFB1. AFB1 reductase is mainly responsible for converting AFB1 to AFL, while AFL dehydrogenase is mainly responsible for converting AFL to AFB1. Since the production of AFL may have a dual effect, it is controversial whether it can be used as a detoxification product. AFL is considered a form of detoxification in fish, combined with glucuronic acid for excretion through bile and feces [44]. Unfortunately, as a storage form of AFB1, AFL can be converted back to AFB1 and continue to be metabolized into AFBO, prolonging the half-life of AFB1 and causing chronic toxicity in AFB1-sensitive animals [45]. Lozano and Diaz (2006) and Murcia and Diaz (2020) also seem to have reached conflicting conclusions. Lozano and Diaz (2006) found that the levels of AFL and AFB1-8,9-dihydrone (AFB1-dhd) produced by turkeys and ducks were 1.97–4.09 times and 1.77–3.15 times, respectively, that of chickens. Because turkeys and ducks are more sensitive to AFB1 than chickens, the author believes that AFL and AFB1-dhd may also have certain toxicity. The large amount of AFL is one reason for the high sensitivity of ducks to AFB1 [46]. Murcia and Diaz (2020) found that chicken AFB1 reductase had the greatest activity, while AFL dehydrogenase showed only minor differences among four poultry (chicken, quail, turkey, and duck). The order of the AFB1 reductase/AFL dehydrogenase ratio from high to low is chicken > turkey > quail > duck [47]. This is inversely proportional to the known sensitivity of poultry to AFB1. Therefore, Murcia and Diaz (2020) believe that AFL is not a toxic metabolite but a detoxified form of AFB1 in poultry. Chickens can convert more AFB1 into AFL, thereby reducing the production of AFBO, which is one of the reasons why chickens are relatively insensitive to AFB1.

Comparing the two studies, we found that there may be two reasons for the cognitive differences in the effects of AFL: the specific content and level of doses were different. First, Lozano and Diaz (2006) measured AFB1-dhd and AFL levels in microsomes and the cytoplasm. They found that although ducks are very sensitive to AFB1, the amount of AFBO produced by their liver microsomes is not high. Murcia and Diaz (2020) studied the kinetic parameters (Vmax, KMr, and CLint) of AFB1 reductase and AFL dehydrogenase. The article stated that “chicken AFB1 reductase has the greatest activity so that it can produce most efficiently.” AFL is the only speculation and has not been verified by further experiments. Another difference is the dose of AFB1. Lozano and Diaz (2006) used 128 μM AFB1 for processing. Murcia and Diaz (2020) found that when 0–9 μM AFB1 was used to treat chicken liver microsomes, AFB1 reductase had the greatest activity, while the difference in AFL dehydrogenase was small. When AFB1 is greater than 30 μM, the
AFB<sub>1</sub> reductase and AFL dehydrogenase activities of ducks and turkeys are much higher than those of chickens, indicating that the mutual transformation of AFL and AFB<sub>1</sub> in ducks and turkeys is more active than that of chickens. This can be partially corroborated by the results of Lozano and Diaz (2006), who found that turkeys and ducks produce higher AFL levels than chickens. Based on the possible dual effects of AFL production and previous studies, we speculate that poultry can tolerate a certain concentration of AFL, so converting AFB<sub>1</sub> to AFL within a certain level can prevent the epoxidation of AFB<sub>1</sub> into toxic AFBO, thereby alleviating the toxicity of AFB<sub>1</sub>. Due to the high activity of AFB<sub>1</sub> reductase and AFL dehydrogenase in ducks and turkeys, when too much AFL is transformed and stored, its toxicity will also be toxic to tissues and cells. In past studies, on the metabolic activation and metabolic detoxification of AFB<sub>1</sub>, the function and dual effects of AFL were often overlooked. Therefore, future research on the role of AFL in the metabolic process of AFB<sub>1</sub> is a potential breakthrough point.

3.4. Role of AFB<sub>1</sub>-dhd in the Toxic Effect of AFB<sub>1</sub>. AFL plays the role of storage and buffering. The more AFB<sub>1</sub> converted to AFL, the less AFB<sub>1</sub> will be epoxidized to AFBO. But AFL is not a complete detoxification product. AFBO can be hydrolyzed spontaneously or converted into AFB<sub>1</sub>-dhd under the metabolism of epoxide hydrolase (EPHX). AFB<sub>1</sub>-dhd can interconvert with AFB<sub>1</sub>-dialdehyde under different pH conditions. Unfortunately, as a toxic metabolite of AFB<sub>1</sub>, AFB<sub>1</sub>-dialdehyde and AFB<sub>1</sub>-dhd both cause oxidative stress and react with lysine residues in proteins [35], resulting in cytoxicity [48]. The study sorted the ratios of AFB<sub>1</sub>-dhd/AFB<sub>1</sub>-monoalcohol produced in the order of duck > quail > turkey > broiler. Ducks can produce large amounts of AFB<sub>1</sub>-dhd, and the ratio of AFB<sub>1</sub>-dhd/AFB<sub>1</sub>-dialcohol is more than 100 times higher than that of broilers. Ducks may not be able to metabolize AFB<sub>1</sub>-dhd into nontoxic AFB<sub>1</sub>-diethanol efficiently; so, AFB<sub>1</sub>-dhd may cause protein damage through conversion to AFB<sub>1</sub>-dialdehyde (see Figure 2). AFAR can continue to convert AFB<sub>1</sub>-dhd into AFB<sub>1</sub>-C<sub>6</sub>-monoalcohol and AFB<sub>1</sub>-C<sub>8</sub>-monoalcohol [49]. AFAR can continue to catalyze them to form the final AFB<sub>1</sub>-diethanol (see Figure 2). AFB<sub>1</sub>-diethanol cannot bind to proteins; so, it is a complete detoxification product. AFAR has been shown to play an important role in alleviating the toxicity of AFB<sub>1</sub> in poultry [50]. The low activity of AFAR may be an important reason for the high sensitivity of ducks to AFB<sub>1</sub>, [35]. If individuals with high AFAR activity can be genetically selected from ducks and turkeys, it may be possible to improve the tolerance of ducks and turkeys to AFB<sub>1</sub> to a certain extent through genetic breeding. AFAR activity in liver microsomes increases with age in turkeys [22]. Notably, AFAR enzyme activity is not the only thing that affects the production of AFB<sub>1</sub>-dhd. GSTs can reduce the production of AFB<sub>1</sub>-dhd by combining with AFBO and further affect the production of AFB<sub>1</sub>-dialdehyde (see Figure 2). In pigs and rats, the AFAR enzyme’s inhibitory effect on AFB<sub>1</sub> toxicity is far less than that of GSTs, and no relationship between AFAR and mammalian tolerance to AFB<sub>1</sub> has been found [51]. Therefore, mammals may mainly have a detoxification effect on AFB<sub>1</sub> by GSTs. In short, ducks may metabolize to produce and store AFL, but they cannot bear and convert more AFB<sub>1</sub> into AFBO and AFB<sub>1</sub>-dhd, resulting in their high sensitivity to AFB<sub>1</sub>. However, more research is needed to determine the metabolic fate of AFL and AFB<sub>1</sub>-dhd in ducks.

Different aquatic animals have different sensitivity to AFB<sub>1</sub>. Rainbow trout are very sensitive to AFB<sub>1</sub>, while catfish are less sensitive to AFB<sub>1</sub>. The liver microsomes of rainbow trout have the highest affinity for AFB<sub>1</sub>, CYP2K1 can quickly and efficiently produce a large amount of AFBO, and the enzyme content is more than five times that of catfish [52]. The activity of AFB<sub>1</sub> reductase and the binding efficiency of GST and AFBO are also very low [51]. In contrast, the study found that due to the low content of CYPs in the catfish liver, the efficiency of CYPs in catalyzing the epoxidation of AFB<sub>1</sub> is lower than that of rainbow trout. Moreover, AFB<sub>1</sub> reductase uses AFB<sub>1</sub> to produce AFL faster than CYPs metabolize AFB<sub>1</sub> to produce AFBO. Catfish quickly excrete AFL for detoxification [44]. The process of aquatic animals’ metabolism of AFB<sub>1</sub> and the reasons for the differences in sensitivity can help us understand the mechanism of differences in the sensitivity of different animals to AFB<sub>1</sub> and inspire us to explore the reasons for the high sensitivity of ducks to AFB<sub>1</sub>. This section further supplements and discusses possible other toxicological mechanisms of poultry to AFB<sub>1</sub>. The possible key metabolic pathways of AFB<sub>1</sub> in poultry are shown in Figure 2. Based on previous studies on the sensitivity of different poultries to AFB<sub>1</sub>, there is still a lack of research on the relationship between mutual transformation products (like AFL, and AFL and AFB<sub>1</sub>-dhd and AFB<sub>1</sub>-dialdehyde) and metabolic detoxification enzymes (like GSTs and AFAR) in different poultries.

4. Exogenous Substances Alleviate the Toxicity of AFB<sub>1</sub>

Many methods have been used to reduce the damage of AFB<sub>1</sub> to animals. We can control the level of AFB<sub>1</sub> in the feed from the source by cultivating crops resistant to *Aspergillus* and taking appropriate transportation and storage measures [53]. Controlling the quality of feed and storage conditions can effectively reduce AFB<sub>1</sub> ingested by animals. If aflatoxins already exist in feed, physical, chemical, and biological detoxification can remove or reduce toxicity [11]. Vitamin E and yeast selenium can reduce the adverse effects of AFB<sub>1</sub> on duck growth performance, immune function, and tissue structure [54]. Grape seed proanthocyanidin extract has protective effects against aflatoxicosis caused by 1 mg/kg AFB<sub>1</sub> in broiler chickens [55]. It is an effective strategy to reduce the toxicity of AFB<sub>1</sub> by inhibiting the activity of CYPs and/or enhancing the activity of GSTs. Isoimperatorin can inhibit the activity of some CYPs and induce the activity of some GSTs on rat liver cancer cells, thereby reducing the cytotoxic effect of AFB<sub>1</sub> [56]. Oltipraz can inhibit the activity of CYP1A2 and CYP3A4, leading to the reduction of AFB<sub>1</sub> metabolites in human primary hepatocytes [57]. Butylated hydroxytoluene (BHT) mainly reduces the production of AFBO by regulating phase I related to AFB<sub>1</sub> metabolism in the liver of turkeys, such as competitively inhibiting the effect.
The possible key metabolic pathways and potential regulatory points of aflatoxin B₁ (AFB₁) in poultry (four points), modified from Murcia and Diaz (2020). ⊛ AFB₁ is metabolized by CYPs into AFBO. ⊜ AFBO combines with GSH to form a detoxification compound. ⊟ As the storage form of AFB₁, AFL can be transformed with AFB₁. ⊥ AFBO removes epoxidation and initially forms AFB₁-dhd, which is finally reduced to AFB₁-dialcohol under the action of AFAR.

of CYP1A5 and inducing CYP3A4 to metabolize AFB₁ into AFQ₁ for detoxification [58, 59]. Curcumin has vast therapeutic potential because of its antioxidant, anti-inflammatory, and antiproliferative properties [60]. Curcumin reduces the toxicity of AFB₁ to chicks by inhibiting the gene expression of CYP1A1, CYP1A2, CYP2A6, and CYP3A4 [3]. Sulfuraphane can inhibit the activity of CYPs in the human liver, thereby reducing the formation of AFBO in liver cells and preventing AFB₁-induced cancer [61]. Marine algal polysaccharides can upregulate the gene expression of GSTA3, GPx1, CAT1, and GSTT1 in the bursa of fabric in broilers [62]. Fisetin can enhance various antioxidant enzymes, attenuate the oxidative stress-inflammatory pathway induced by AFB₁, and reduce the risk of liver cancer [63]. Fucoxanthin relieves liver and kidney damage caused by AFB₁ by reducing oxidative stress, DNA damage, and inflammation [64].

These studies indicate that exogenous substances can inhibit or induce metabolic enzyme activity in the body, change the metabolic rate and metabolic pathways of exogenous toxins, and alleviate the animal’s oxidative stress state, reducing the harmful effects of AFB₁. Nevertheless, most of the research model is “exogenous material effect test → mechanism analysis”, which causes blindness in selecting effective exogenous regulators. Therefore, studying the mechanism of ducks’ high sensitivity to AFB₁ can guide the selection of exogenous regulators and reduce blindness. This has important theoretical significance and good economic value.

5. Summary

The activation and detoxification of AFB₁ in poultry involve multiple metabolic pathways. In addition to the activation of CYPs in phase I and the detoxification of GSTs in phase II, there may be other metabolic pathways of AFB₁, such as the mutual conversion of AFB₁ and AFL, the production of AFB₁-dhd, and the enzymatic activity of EPHX and/or AFAR. In summary, the high sensitivity of ducks to AFB₁ may be caused by CYPs producing too much AFBO and AFL and lack of effective GSTs and AFAR to further metabolize harmful AFBO and AFB₁-dhd. In the future, the key control points of AFB₁ metabolism in ducks should be clarified, such as enhancing the activity of GSTs and AFAR, to guide the selection of exogenous regulators.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors’ Contributions

Kuntan Wu contributed to the writing—reviewing, editing, and investigation. Minjie Liu contributed to the writing—original draft preparation and conceptualization. Huanbin Wang performed the data curation. Shahid Ali Rajput performed the methodology. Yajing Shan performed the visualization and software. Desheng Qi and Shuai Wang contributed to the supervision. All authors read and approved the final manuscript.

Acknowledgments

This research was funded by the National Key Research and Development Program of China (project no. 2016YFD0501207) and the National Natural Science Foundation of China (project no. 31772635).

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