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Chapter 8

Control of Aflatoxicosis in Poultry Using Probiotics and Polymers

Bruno Solis-Cruz, Daniel Hernandez-Patlan, Billy M. Hargis and Guillermo Tellez

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Abstract

An important approach to prevent aflatoxicosis in poultry is the addition of non-nutritional adsorbents in the diet to bind aflatoxin B1 (AFB1) in the gastrointestinal tract. These adsorbents are large molecular weight compounds that are able to bind the mycotoxin, forming a stable complex adsorbent-mycotoxin, which can pass through the gastrointestinal tract. In this chapter, we evaluate the use of polymers and probiotics to reduce AFB1 toxic effects in poultry. Our results on the efficacy of polymers and probiotics in sequestering mycotoxins are highly promising, although this field is still in its infancy and further research is needed. Furthermore, *in vivo* studies are needed to confirm the effectiveness of these materials against AFB1 toxic effects, since results in the past have indicated that there is great variability in the efficacy of adsorbing materials *in vivo*, even though the compounds may show potential adsorption capacity of the mycotoxin *in vitro*.

Keywords: aflatoxins, chickens, polymers, adsorption, probiotics

1. Introduction

Mycotoxins are low molecular weight compounds produced as secondary metabolites by filamentous fungi contaminating crops in the field or warehouses when environmental conditions of temperature and humidity are adequate. These metabolites have no biochemical relevance to fungal growth or development, and they constitute a chemically and toxicologically heterogeneous group, which are together only because they can cause diseases, including death, to human beings and other animals even at low concentrations [1].

Currently, more than 400 different mycotoxins are known, but only six are currently considered to be of worldwide importance, and aflatoxins are the most toxigenic and investigated mycotoxins worldwide because their natural occurrence can cause serious economic losses and health problems [2, 3]. In terms of toxicity and occurrence, aflatoxin B1 (AFB1) is the most important mycotoxin due to its hepatotoxic and hepatocarcinogenic effects, which can result in immunosuppression, anorexia with reduced growth rate, decreased egg production, reduced reproductivity, poor feed utilization, anemia, hemorrhage, and increased mortality [4, 5]. Furthermore, intoxication with AFB1 has been linked to other severe effects such as teratogenesis, carcinogenesis, and mutagenesis [6].

Due to the severe and harmful effects of AFB1, many methods to reduce its toxic effects have been proposed. The first and best attempt to prevent the effects of AFB1 is to minimize its production through good agricultural practices (GAP), including cultivating practices in fields as well as harvest, transport, and storage conditions [7, 8], all these steps are under GAP. However, since prevention is not always possible, decontaminating and/or detoxifying methods have been gaining attention as an alternative to reducing AFB1 contamination of feed and grains. Methods of detoxification can be physical, chemical, or biological treatments of contaminated feed or grains, and they can be as simple as the physical separation through screening, classification, and selection of damaged grains or as complex as gamma irradiation or chemical methods using ammonia, ozone, hydrogen peroxide, or some acids and alkalis [9–14]. Nevertheless, many of these methods to detoxify aflatoxin-contaminated feed are not currently available because they cannot be applied on a large scale and in a cost-effective manner or because many of them are impractical, ineffective, or potentially unsafe.

Another approach to prevent aflatoxicosis in animals is the addition of adsorbents in the diet for binding aflatoxin in the gastrointestinal tract so that these compounds impede its adsorption in the intestine [15]. Adsorbents have been recurrently used because of their economic feasibility and suitability for nutritional perspective [16]. Many studies have demonstrated that aluminosilicates, mainly zeolites, hydrated sodium calcium aluminosilicate (HSCAS), and aluminosilicate-containing clays, can effectively reduce aflatoxins toxicity to animals; being these inorganic materials, the most thoroughly studied adsorbents [17–21]. Alternatively, both carbon-based organic polymers and synthetic polymers have been tested, and some of them are currently on the market [17, 22]. Even though the cost of these polymers could be the limiting factor for practical applications, their use can help to solve the problems related with the use of aluminosilicates and clay adsorbents, such as binding preferly just to aflatoxins, the possibility to adsorb important micronutrients, and the risk of natural clays to be contaminated with dioxins [7, 23]. Nowadays, there are some highly promising research on the effectiveness of synthetic and organic polymers in adsorbing aflatoxins, although this field is still under developing and it needs more *in vitro* and *in vivo* research [24].

On the other hand, biological methods to prevent aflatoxicosis have also been evaluated showing promising results [25–28]. Many microorganisms, including bacteria, yeasts, molds, actinomycetes, and algae, have been tested for their ability in the control of aflatoxin contamination, mainly through adsorption and degradation [29, 30]. Among the bacteria tested, probiotics have been identified as a good option to reduce the availability of aflatoxins *in vitro*. Additionally,

probiotic bacteria have shown numerous beneficial health effects, which make them even more suitable additives to food and feed [25, 31–33].

2. Biological importance of AFB1 in poultry

Poultry species are probably the most sensitive food-producing animals to AFB1 toxic effects, and small amounts of it severely damage animal health and the profitability of the productive system, which results in substantial annual economic losses to producers [6, 34–39].

However, there are also differences in terms of susceptibility to AFB1 among poultry species, which could be due to differences in hepatic metabolism of AFB1 in these species. According to comparative toxicological studies, ducklings and turkey poults are the most sensitive species to AFB1, followed by goslings and young pheasants with intermediate sensitivity, and finally, the chicks showed to have relative resistance to AFB1 injury [40]. Toxicity and carcinogenicity of AFB1 occur after its bioactivation by the cytochrome P450 (CYP450) mixed function oxidase system, resulting in a highly reactive AFB1 8,9-epoxide (AFBO), which forms covalent adducts with cellular macromolecules such as DNA, RNA, protein constituents, and some enzymes [41–44]. Since metabolic activation of AFB1 to AFBO by CYP450 is especially efficient in poultry species [45], they are extreme sensitivity to the toxic effects of AFB1. Another possible reason which may also explain the differences in susceptibility of poultry species is the variation in phase II biotransformation enzymes, such as glutathione S-transferase (GST), that catalyze a conjugation reaction of AFBO with endogenous glutathione (GSH). Although avian species are highly efficient in producing AFBO, they are not able to conjugate it effectively with GSH, which indicates that they have low GST activity [46, 47].

The most noticeable effect of AFB1 on poultry is the impair of all important productive parameters, including body weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, male and female reproductive performance, and an increased mortality [35, 48, 49]. These alterations in the productive parameters are the result of the physiological effects of AFB1 consumption, of which liver damage is the most notorious, characterized by its enlargement, pale yellow coloration, petechial hemorrhages and hematomas on the surface, usually accompanied with proliferation of biliary ducts and depletion of lymphoid organs [50–52]. However, for poultry industry AFB1 contamination and consumption are important because of its ability to decrease resistance to common infectious diseases, including parasitic, bacterial, and viral infections, due to depression of the humoral and cellular immune responses [53–57].

3. Microbiological control of AFB1

To date, many physical and chemical methods have been used to detoxify AFB1; however, only a few of these methods are in practical use, probably due to difficulties in complying with the FAO requirements: reduction of AFB1 without residual toxicity, guarantee of nutritional

values, and no modification of food or feed properties [58, 59]. Since cost-effective methods to detoxify mycotoxin-contaminated grains and foods are urgently needed to minimize potential losses to the farmer and toxicological hazards to the consumer [60], finding of new and suitable methods for AFB1 decontamination has become a primary need.

In this sense, microbiological control approach has taken strength in the field of research to control AFB1. Researchers have focused on biological treatments for detoxification mainly through two mechanisms: adsorption and degradation, both of which can be achieved by biological systems such as bacteria, yeasts, molds, actinomycetes, and algae [61].

Biological adsorption can occur either by attaching the AFB1 to the cell wall components of the microorganisms or by active internalization and accumulation. Also, dead microorganisms can absorb AFB1, and this phenomenon can be exploited in the creation of biofilters for fluid decontamination or probiotics to bind and remove the AFB1 from the intestine [62]. However, biological adsorption mechanism is naturally reversible, and AFB1 may be easily released, so that it is necessary to search for novel approaches to overcome these drawbacks, as for example the combination of mineral and biological adsorbents to improve their effectiveness [63].

On the other hand, microbiological biodegradation is performed by either extracellular or intracellular enzymes, so the degradation is generally permanent and irreversible which can alter, reduce, or completely eradicate AFB1 toxicity [30]. Nevertheless, modification of AFB1 structure can result in other molecules, such as aflatoxicol (AFL), also with potential toxic effects [64]. Thus, further knowledge is needed on the identification, quantity, and toxicity of degradation metabolites prior to the potential applications of biological treatments [59].

Microbiological control seems to be becoming one of the most promising approaches for AFB1 control; since the last four decades, the use of microorganisms is one of the well-known strategies for the management of AFB1 in foods and feeds. These methods of bioadsorption and biodegradation are being actively studied and can be a highly promising choice because they are efficient, specific, and environmentally friendly [65–68].

4. Use of probiotics to prevent AFB1 toxic effects in poultry

Microbiological control of AFB1 is still considered as a promising area in research; so recently, these methods have attracted researcher's attention due to their easy usage and affordable processes [69]. However, since the use of microorganisms is expected to be safe both for animal health and for the production of innocuous livestock products, there are still many microorganisms that cannot be directly employed in the food or feed directly. In the last decades, research to find microorganisms for AFB1 control has focused on testing, screening, and choosing those strains that have demonstrated their effectiveness not only to reduce or even suppress AFB1 toxicity but also to be Generally Regarded as Safe (GRAS) [70, 71].

There are several microorganisms that have been shown to be effective in preventing and controlling the toxic effects of AFB1; among them, probiotic bacterial strains are some of the

most studied, due largely to their GRAS character and because they have shown to have several potential applications against AFB1 both *in vitro* and *in vivo* [72–75]. Probiotics are living microorganisms that when administered in adequate amounts confer a health benefit to the host directly or indirectly through the maintenance of the microbial balance in their digestive tract [65, 76]. Several bacterial genera have been used as probiotics in livestock, including many species of *Bacillus*, *Bifidobacterium*, *Enterococcus*, *E. coli*, *Lactobacillus*, *Lactococcus*, and *Streptococcus*, although some species of molds and yeasts, such as *Aspergillus*, *Candida*, and *Saccharomyces*, have also been used [77, 78].

In poultry industry, probiotics have been reported to have a beneficial effect on performance, modulation of intestinal microflora and pathogen inhibition, intestinal histological changes, immunomodulation, certain hematobiochemical parameters, improving sensory characteristics of dressed meat, and promoting microbiological meat quality [79, 80]. In addition, probiotic bacteria may possess antimutagenic and anticarcinogenic activity. The mechanisms of these activities remain unclear; however, alteration of fecal bacterial enzyme activities associated with conversion of promutagens and procarcinogens to ultimate carcinogens and binding of dietary mutagens and carcinogens has been proposed [81].

Three possible mechanisms have been proposed by which probiotics can counteract the toxic effects of AFB1: (1) competing with aflatoxigenic mold strains for space, occupying the same ecological niche or using nutrients, and thus reducing AFB1 biosynthesis; (2) encouraging AFB1 metabolic degradation by enzymes, or (3) impeding its intestinal absorption by AFB1 binding onto the cell walls of the probiotics strains.

It has been suggested by *in vitro* studies that probiotics can inhibit AFB1 production through releasing metabolites to the media, such as organic acids, bacteriocins, and even hydrogen peroxide, which may interfere with AFB1 biosynthesis [82, 83]. Other alternative could be the reduction or inhibition in the growth of aflatoxigenic mold strains caused by a decrease in pH of the media and/or a nutrient competition of the culture media, which could also have contributed to the removal of AFB1 [84–87]. In **Figure 1**, it is shown how some probiotics from the lactobacilli strains can decrease both AFB1 production and the growth rate of an aflatoxigenic mold strain.

Although several bacterial strains have been used as biocompetitive agents of aflatoxigenic mold strains, some of them become inactive under extreme conditions of humidity and temperature, so that not all probiotic strains are ideal for this application. In this sense, studies on the prevention of AFB1 contamination using highly competitive non-toxigenic strains of *A. parasiticus* and *A. flavus* have shown certain advantages, which implies that these mold strains may be potentially useful as agents directed at competitively excluding toxigenic strains [88].

The other mechanism that the probiotics have to counteract the toxic effects of AFB1 is through its metabolic degradation or biodegradation, which can be defined as the degradation or enzymatic transformation of the mycotoxin to less or non-toxic compounds. Biodegradation using microorganisms or their enzymes is one of the most studied strategies for AFB1 management; this method has been actively studied and can be a highly promising choice, since it is efficient, specific, and environmentally friendly to reduce or eliminate the possible contaminations of

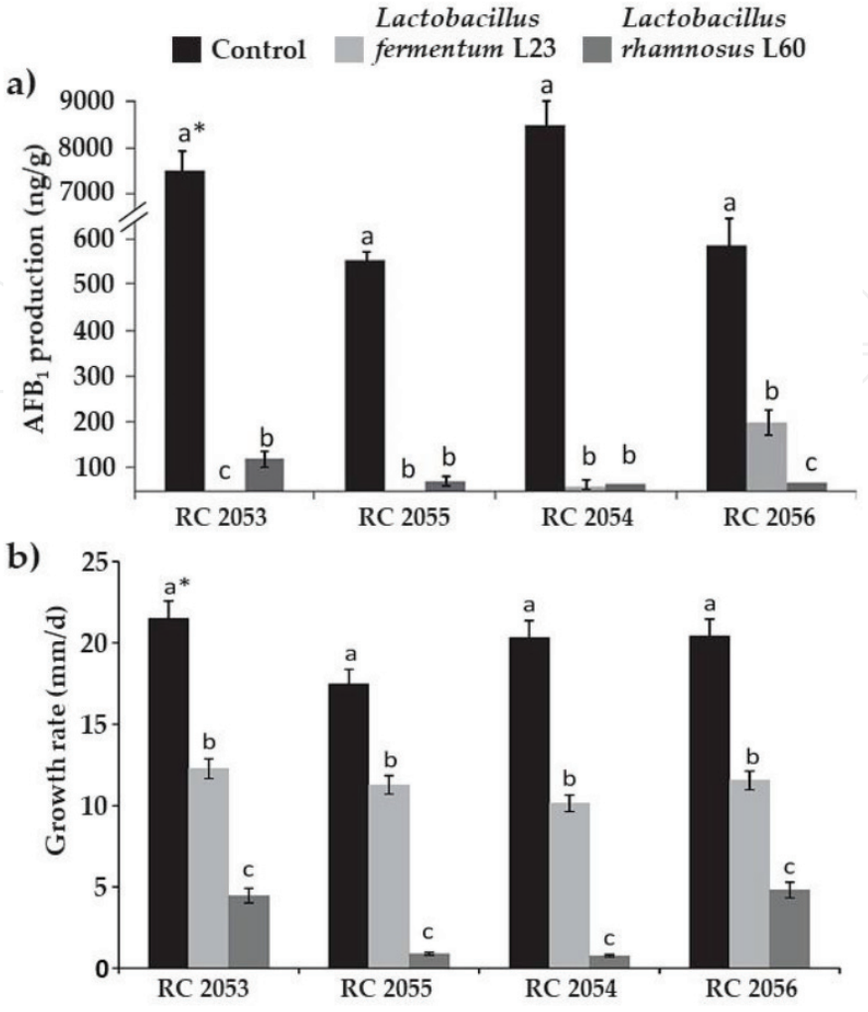

Figure 1. Effect of lactobacilli strains on: (a) the production of AFB1 and (b) the rate growth by *Aspergillus* section *Flavi*. Mean values based on quadruplicate data. * Mean with a letter in common is not significantly different according to Tukey's test ($p < 0.05$) (modified from [83]).

AFB1 under mild conditions, without using harmful chemicals and without significant impairment of the nutritive value or palatability of the detoxified food or feed [68].

Studies on microbial degradation of AFB1 involve the use of microbial catabolic pathways, which act on one of the two key sites influencing its toxicity and potency, shown in **Figure 2**. The first site is the double bond in position 1,2 of the furofuran ring [41], and the second reactive group is the lactone ring in the coumarin moiety [89]. AFB1 is usually detoxified to a less toxic compound by opening the lactone ring, altering the coumarin structure, but it can also occur by removing the double bond from furan ring when there is a scission on it [2, 90, 91]. It is known that opening the lactone ring abolishes or decreases the fluorescence spectrum of AFB1; however, the cleavage of the furofuran ring does not change its fluorescence properties [92].

For AFB1 metabolic degradation, several microbial isolates have been studied and reported with different levels of degradation capacities, including bacteria and fungi strains [94–101];

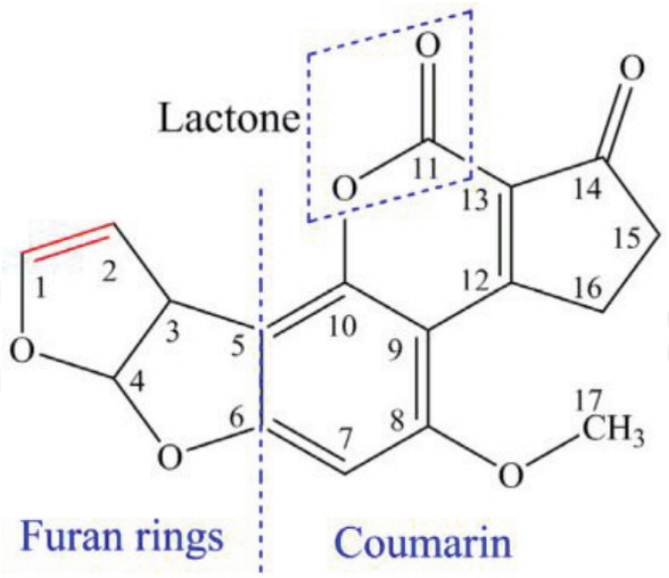

Figure 2. Chemical molecular structure of AFB1, showing the two key sites responsible of its toxicity (taken from [93]).

however, for the fungi species, limitations such as long degradation time, non-adaptability to typical food fermentations, and culture pigmentation reduced their potential application in AFB1 detoxification [97], besides the use of fungi species is not economical because of the extraction process and lengthy incubation time [102]. Moreover, some of these fungi strains with degradation potential may also produce AFB1 under varying conditions [103].

One of the first studies in this area was carried out in the 1960s, when it was evaluated the ability of about 1000 types of microorganisms to degrade aflatoxins [61]. Since then, many other studies have been done with several bacterial genera and strains; being the lactic acid bacteria (LAB), the most studied to detoxify AFB1; nevertheless, the ability of LABs to detoxify AFB1 has been attributed to their strong affinity and capacity to adsorb the toxin rather than for their degradation abilities [75, 81, 104–106].

AFB1 degrading activity has been found in other bacteria genera, such as *Mycobacterium fluoranthenivoran*, *Nocardia corynebacterioides* (formerly *Flavobacterium aurantiacum*), *Rhodococcus erythropolis*, *Stenotrophomonas maltophilia*, *Pseudomonas*, as well as *Bacillus licheniformis* and *B. subtilis* [70, 71, 97, 107–110], which have demonstrated that their biodegradation activity is from enzymatic nature. For example, *B. subtilis* JSW-1, a bacterium isolated from soil samples, is able to degrade almost 70% of AFB1 within 72 h, as shown in **Figure 3**, and its degradation activity was likely due to the extracellular enzymes [26]. In other study, biological degradation of AFB1 by *Rhodococcus erythropolis* was evaluated in liquid cultures, in which dramatic reduction of AFB1 was observed after 48 and 72 h of incubation with just 17 and 3–6% of residual AFB1, respectively [97]. The ability to effectively biotransform AFB1 by *Myxococcus fulvus* has also been demonstrated. This bacterial isolate from deer feces was able to biotransform AFB1 by 80.7% after 72 h [111].

Although probiotic bacterial strains are more desirable for AFB1 degradation, the use of whole cultures has less potential for large-scale utilization in the industry, so the use of fractions (cells

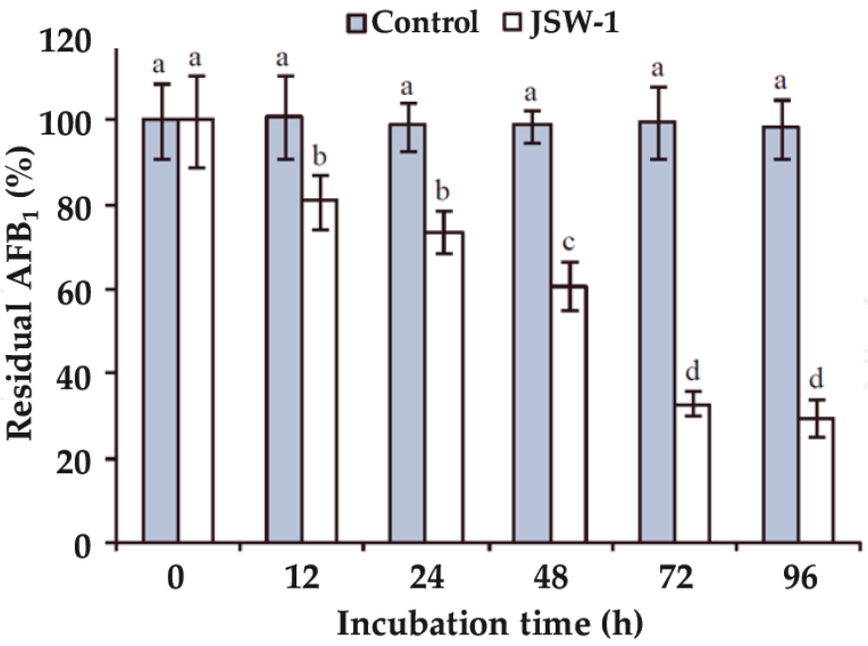

Figure 3. Time course of *in vitro* AFB1 degradation by *B. subtilis* JSW-1 at 30°C for 12, 24, 48, 72, and 96 h in the dark. The initial concentration of AFB1 was 2.5 mg/mL. Values represent the mean ± SD (n = 3). Values with different letters indicate significant differences ($p < 0.05$) among them (modified from [26]).

or lysates) may be convenient, since they are substrate specific, effective, environmentally friendly, and possess better utilization in the food and feed industry [112].

In literature, there are many studies of AFB1 biodegradation carried out in laboratory conditions with many probiotic strains; however, the information in livestock and poultry about the effect of probiotics on AFB1 detoxification is very limited, especially in poultry science. This is important because *in vitro* studies are not always good indicators of the *in vivo* responses, since there are physiological parameters, such as pH, peristaltic movements, and gastric and intestinal secretions affecting their *in vivo* behavior. This can be observed in studies carried out with the genus *Bacillus* spp., of which some strains have been identified as GRAS organisms with probiotic properties in humans and animals as direct fed microbials (DFM). In the *in vitro* study, 3 of 69 *Bacillus* spp. candidates, which were evaluated, showed ability to biodegrade AFB1, based on growth as well as reduction of fluorescence and area of clearance around each colony [70]. However, when the biodegradation potential of these selected *Bacillus* spp. was tested in broiler chickens, no beneficial performance effects were showed. In addition, no significant performance differences were observed when compared with their respective control diets [113]. Therefore, there is still missing research to evaluate the effect of AFB1 degrading probiotics on growth performance, digestibility, immune function, and toxic residues in tissues and excreta in livestock production animals.

The other mechanism that the probiotics have to counteract the toxic effects of AFB1 is through its physical adsorption, which is in fact the most commonly used technique for reducing exposure to AFB1 [114]. It has been demonstrated that AFB1 is absorbed into the enterocytes by passive diffusion so, after its oral ingestion, AFB1 is efficiently absorbed in the intestinal

tract, being the duodenum the major site of absorption [115]. If the AFB1 is physically linked to the probiotic microorganism, its bioavailability is decreased, and therefore AFB1 uptake and its access to systemic circulation are also diminished. Adsorption is a physical process, in which the cell wall of microorganism binds the toxin by non-covalent weak bonds and some electrostatic attraction. This interaction appears to occur predominantly with polysaccharides, peptidoglycan, and teichoic or lipoteichoic acids in the cell wall [116–118].

In vitro adsorption of AFB1 by probiotics has been described as a fast and reversible process, which is affected for many factors such as strain, toxin dose, temperature, pH, and microorganism concentration [72, 104, 118–120]. It has also been demonstrated that viability of some probiotic strain does not affect their absorption ability; thus, viable, heat-killed, and acid-killed cells respond in a similar manner [118, 121].

Several studies have been done in optimal laboratory conditions with several strains of probiotic microorganisms tested for their capacity to adsorb AFB1 and have been reported a wide range of genus, species, and strain-specific binding capacities [75, 81, 104, 116, 122–125], being the LABs and yeasts such as *Saccharomyces cerevisiae* those that have demonstrated the greatest ability to remove AFB1 by its adsorption [126]. Such is the case of *Lactobacillus rhamnosus* GG and *Lb. rhamnosus* LC-705, which have demonstrated to be very effective for removing AFB1, being able to remove up to 80% of the toxin instantly [104, 127]. On the other hand, yeasts have been reported to have similar mechanism as LAB in binding to AFB1 as a means of detoxification [68, 126], with studies that have shown that some strains of *S. cerevisiae* can adsorb up to 90% of AFB1 [123, 128].

There is strong evidence in literature that some specific probiotics can adsorb AFB1 *in vitro*, but only limited information is available on adsorption in poultry *in vivo*. These *in vivo* studies are really important since *in vitro* studies have shown that there are relevant physiological conditions that the microorganisms encounter during their passage through the gastrointestinal tract, such as pH, intestinal mucus, and presence of bile, which modify the AFB1 adsorption and the stability of the AFB1-microorganism complex, either positively or negatively [122]. Although not many probiotic strains have been tested *in vivo*, the studies that have been conducted in poultry showed good results, such as in the *in vivo* study using the chicken duodenum loop technique, in which probiotic strain GG of *L. rhamnosus* removed as high as 54% of the added AFB1 and reduced its intestinal adsorption by 73% [73]. In this study, there was a difference in adsorption capacity when these strains were incubated *in vitro*, being the reduction of AFB1 even higher *in vivo* when compared to its adsorption *in vitro*. *Bacillus* probiotics have also been proved to remove or reduce AFB1 adsorption in the gastrointestinal tract at *in vivo* and *in vitro* conditions, showing the positive impact of these bacteria in preventing the harmful effects of aflatoxin in poultry with regard to performance, serum biochemistry, and immune responses [69]. However, when the capacity of *Bacillus* and *Lactobacilli* strains to control the stressful effects caused for AFB1 on chickens was compared, the *Lactobacilli* abilities resulted to be higher. This study shows that these probiotics can control the toxicity of AFB on poultry by improving humoral and cellular immune function, serum biochemical parameters, the process of protein synthesis, and reducing the anti-nutritional effects of AFB1 [65]. In a recent study, the effect of lactic acid bacteria and HSCAS on

detoxification of AFB was evaluated in broiler chickens. The results showed that LAB or HSCAS supplementation improved the growth performance, digestibility, and immune function of birds, reducing deleterious effects and tissue residues of AFB1; however, the effect of LAB resulted to be more effective than HSCAS, which indicates a possible mechanism of biodegradation of the toxin by the probiotics [129].

5. Use of polymers to prevent AFB1 toxic effects in poultry

As it was mentioned in Section 1, an important approach to prevent aflatoxicosis in livestock and poultry is the addition of non-nutritional adsorbents in the diet to bind AFB1 in the gastrointestinal tract, reducing its bioavailability, which leads to a reduction of mycotoxin uptake as well as distribution to the blood and the target organs. These adsorbents are large molecular weight compounds that are able to bind the mycotoxin, forming a stable complex adsorbent-mycotoxin, which can pass through the gastrointestinal tract of the animals without dissociating the AFB1, to be eliminated via the feces [22].

The efficacy of adsorption appears to depend on the chemical structure of both the adsorbent, the mycotoxin, and the feed components. The physicochemical properties of the adsorbents such as total charge, charge distribution, size of the pores on the surface, surface area, iodine number, methylene blue index, and pH take on an important function in binding effectively. On the other hand, the properties of the adsorbed mycotoxins, like polarity, solubility, size, shape, charge distribution, and dissociation constants, also play a significant role. It has also been mentioned that the high fiber content of the feed substrate increased the mycotoxin affinity to adsorbent [17, 18].

Even though clay minerals and aluminosilicate materials have been tested and recognized for their ability to bind AFB1 successfully [130, 131], the main risk of using them in animal feed is that they can also adsorb some feed vitamins and minerals, decreasing their utilization by animals [132, 133]. Another risk is that clays can release toxic components or elements bound to them, as heavy metals or dioxins, which can be released in the intestine of animals and accumulated in animal organs [134, 135].

Facing the problems of the use of clay and aluminosilicate adsorbents, other types of binders have been investigated in the search for new adsorbent materials such as organic binders or biopolymers and synthetic polymers [17, 112]. Both kind of polymers are large molecules that are composed of many monomers, whose large molecular mass relative to a small molecule produces unique physical properties playing important roles in our society [24]. Just a few synthetic polymers have been evaluated and demonstrated to bind mycotoxins *in vitro* and *in vivo*, such as cholestyramine, divinylbenzene-styrene, polyvinylpyrrolidone (PVP), and its modification polyvinylpolypyrrolidone (PVPP) [7, 17, 18, 112]; nevertheless, from these polymers, only PVP and PVPP have been tested against AFB1 in poultry. *In vitro* studies indicate that PVPP can bind up to 50 mg/kg of AFB1 from feed. On the other hand, *in vivo* studies carried out in broiler chickens demonstrated that PVPP could have ameliorated some serum biochemical and hematological parameters, it might have meliorated the detrimental effects of AFB1 on the immune system, and that the pathological changes were markedly inhibited by

the administration of PVPP in the diet [136–139]. However, the cost of those polymers would be a limiting factor for practical applications.

Biopolymers are generally complex indigestible carbohydrates, non-toxic, biocompatible, and biodegradable, such as cellulose, cellulose, lignin, hemicellulose, glucomannans, peptidoglycans, and chitosan. They have been widely used as a promising biosorbents for the removal of various heavy metal ions and dyes [140], but recently cellulosic polymers and chitosan have been demonstrated to have ability to adsorb AFB1 [24, 141]. According to the *in vitro* results, both cellulosic polymers and chitosan were able to bind other important mycotoxins for poultry industry besides AFB1, which is a clear advantage over inorganic adsorbents since they are very effective in preventing aflatoxicosis, but their efficacy against mycotoxins such as zearalenone, ochratoxin, and trichothecenes is limited [17]. These biopolymers also pose multilayer porous structure filled with openings and channels that provide huge volume per sorbent surface unit, which is favorable in the adsorption process. Concerning to chitosan, different molecular weights, deacetylation degree, and cross-linked degree have to be tested for their AFB1 adsorption properties, because these characteristics might show different adsorptive capacity against this mycotoxin [24].

The results on the efficacy of polymers in sequestering mycotoxins are highly promising, although this field is still in its infancy and further research is needed. Furthermore, *in vivo* studies are needed to confirm the effectiveness of these materials against AFB1 toxic effects, since results in the past have indicated that there is great variability in the efficacy of adsorbing materials *in vivo*, even though the compounds may show potential adsorption capacity of the mycotoxin *in vitro* [22].

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