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# EVALUATION OF THE EFFECTS OF MYCOTOXIN BINDERS IN ANIMAL NUTRITION

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## Abstract

Mycotoxins are small and quite stable molecules which are extremely difficult to remove or eradicate, and which are considered to be a great threat both for human and animal health in global terms. Especially in farm animals, mycotoxins can cause decreased performance, reducing in feed consumption, weakening of immunity system, reproductive disorders, diminished body weight gain and residues on food products of animal origin. The mycotoxins of major concern as feed contaminants that are potentially removable from feed are mainly aflatoxins, ochratoxin A, zearalenone, deoxynivalenol, T-2 and fumonisins. One of the methods for reducing the exposure to mycotoxins is to reduce their absorption and bioavailability by using various mycotoxin binders. The most widely known of these are aluminosilicates like clay, bentonite, montmorillonite, zeolite, aqueus sodium calcium aluminosilicate (HSCAS) and active carbons. Another method is the degradation of mycotoxins into non-toxic metabolites by using indigestable complex carbonhydrates (bacterium and yeast cell walls), enzyme, vitamin, amino-acid and synthetic polymers like cholestralamine, polivinil-polipirrolidon polymers (PVPP). The purpose of this review is to identify the benefits and risky aspects of using toxin binders in animal nutrition and to give some idea about the future of this practice.

**Keywords:** Mycotoxins, Toxin binders, Feed, Livestock

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## 1. Introduction

Mycotoxins, derived from of the word “mykes” meaning mycose and the word “toxin” meaning poison, are low molecular weight secondary metabolites produced by a great variety of mycose, especially fungi. As far as is known; there are over 200 types of fungi producing mycotoxin, and about more than 300 mycotoxin produced by these fungi. Mycotoxins such as aflatoxins, zearalenone, ochratoxin A, fumonisins, trichothecenes including deoxynivalenol and T-2 toxin are the most significant types that have negative effects on the health and performance of all livestock, particularly poultry (Murugesan et al., 2015). The FAO has estimated that worldwide approximately 25% of agricultural commodities are affected by mycotoxins (Jelinek et al., 1989; Chilaka et al., 2017). Mycotoxins that have generated a major risk factor for humans and widely public health. Mycotoxins have different biological effects such as carcinogenic, mutagenic, teratogenic,

oestrogenic, neurotoxic, immunotoxic, etc. (Alçiçek, 2012).

Especially in farm animals, mycotoxins can cause decreased performance, reducing in feed consumption, weakening of immunity system, reproductive disorders, diminished body weight gain and residues on food products of animal origin (Kolosova and Stroka, 2012). Therefore, the potential effects that may be observed in animals following toxic compound intake, can vary from the acute cases such as certain diseases or death to the chronic illnesses such as reduced resistance to pathogens or reduced animal production. However, it has been reported that the main problem observed in the animals fed by food contaminated with mycotoxins is the chronic diseases, including many metabolic, physiological or immunological illnesses caused by the regular intake of toxin with low level, rather than acute problems (Grenier and Applegate, 2013). Biomin® has summarized the primary effects of mycotoxins as shown in the table 1 below (Anonymus, 2018).

**Table 1.** Primary mechanism of action of main mycotoxin groups

| Mycotoxin | Primary mechanism of action |
|---|---|
| Aflatoxin | Binds to guanine (DNA-adduct) after metabolic activation in the liver |
| Trichothecenes | Inhibition of protein synthesis |
| Zearalenone | Binds to mammalian estrogen receptor |
| Ochratoxins | Blocks protein synthesis |
| Ergot alkaloids | Binding to adrenergic, dopaminergic and serotonin receptors |
| Fumonisins | Inhibit ceramide synthase (sphingolipid biosynthesis) |

Due to the frequent occurence of mycotoxins and toxicities, methods to prevent or reduce exposure to these and others are in demand (Bursian et al., 2004). For this, various physical, chemical and biological methods applied. But used of these avaliable methods for the detoxification of feed contaminated with mycotoxins is restricted because of the problems associated with health and safety issues, possible losses in the nutritional quality of treated feeds coupled with limited efficacy and cost implications. An alternative and popular approach to decreasing mycotoxin toxicity in animals is the use of toxin binders as feed additives that can reduction the contamination of feed by mycotoxins and suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action (Kolosova and Stroka, 2012). Depending on their mode of action, these feed additives act either by reducing the bioavailability of the mycotoxins or degrading or transforming them into less toxic metabolites (biotransformation) (EFSA, 2009). According to this toxin binders can be gathered under two grubs.

One of the strategies for reducing the exposure to mycotoxins is to decrease bioavailability of mycotoxins by adsorbents. High molecular weight adsorbing agents prevent the absorption of mycotoxins by reacting with mycotoxins in the gastrointestinal system (aqueous medium), and the resulting complex of adsorbent-toxin is discharged with feces. (Di Gregorio et al., 2014). The most mainly known of these are aliminosilicates (bentonites, montmorillonites, zeolite, HSCAS (Hydrated sodium calcium aluminosilicate), etc.), indigestible complex carbohydrates (cellulose, polysaccharides in the cell walls of yeast and bacteria such as glukomannans and peptidoglycans), actived carbon or charcoal, synthetic polymers such as cholestryamine and polyvinylpyrrolidone (Whitlow, 2006).

Another strategy is the degradation or transformation of them into less toxic metabolites by using biotransforming agents such as bacteria (gram-pozitive anaerobic bacteria, gram-pozitive aerobic bacteria, gram-negative aerobic bacteria), fungi (*Aspergillus spp., Eurotium herbariorum, Rhizopus spp., Penicillium raistricki, Rhinocladiella atrovirens*), yeast (*Trichosporon mycotoxinivorans, Phaffia rhodozyma and Xanthophyllomyces dendrorhous isolates*), enzymes (protease A, pancreatin, carboxypeptidase A, epoxidase, lactonohydrolase) (EFSA, 2009). Mycotoxin binders are supposed to detoxify the contaminated feedstuffs during passage through the digestive tract by adsorbing or degrading the mycotoxins under the pH, temperature and moisture conditions of the digestive tract (Döll and Danicke, 2004).

## 2. Some Mycotoxin Binders Used in Our Country and the World

To be used some of mycotoxin binders (absorbing or biotransforming agents) in livestocks given in the Table 2, 3 and 4. Accordingly, in these tables mentioned about studied mycotoxin, mycotoxin levels, used mycotoxin binders, details on animals, product inclusion, parameters evaluated and effect of used product.

## 3. The Future of Mycotoxin Binders

Mycotoxin binders are being used to prevent the feed containing low levels of mycotoxin from being spoilt and to avoid the negative situations that cause chronic diseases and that reduce the performance in animals. Therefore, these binders are for protecting animal health and animal production rather than providing treatment (Whitlow, 2006).

Five key elements need to be known for a substance to be considered as a binder. These key elements are adsorption capacity, irreversibility (firmly binding to

toxins and difficulty of dissolution), specificity (binding only to toxins without any interaction with the food), safety and the results obtained from in vivo work (Anonymous, 2016).

**Table 2.** Mycotoxin adsorbing agents used in poultry (broiler)

| Mycotoxin | Mycotoxin levels | Product | Product inclusion | Effect of used product | Literature |
|---|---|---|---|---|---|
| Aflatoxin (AF), Ochratoxin, Toxin (T-2), Fuminisin, Zearalenone and Vomitoxin | 7.4 µg/kg, 10.0 µg/kg, 79.8 µg/kg, 700.0µg/kg, 100.0 mg/kg 0.8 mg/kg | Activated charcoal (AC), Modified yeast cell wall extract (esterified glucomannan) or HSCAS | 1 or 2% 0.05 or 0.1%, 0.5% | Addition of 2% AC to moldy corn has increased death ratio and foot problems in broilers whereas other binders have reduced them. It was found that there were no differences between the groups in terms of liver, spleen and tibia weights. | Wang et al., 2006 |
| T-2 | 2 mg/kg | Inorganic, organic or mixed of the adsorbents | 0.2% of each adsorbent | In the study, it was reported that the inorganic and organic absorbents eliminate the adverse effects of T-2 and that the biggest reduction was in the mixed group. | Nešić et al., 2011 |
| Aflatoxin B1 (AFB1) | 200 µg/kg | Magnetic carbon nanocomposite | 0.3% | Adsorbent has reduced the death rate and the liver weight in the chickens fed with AFB1. It has also adsorbed and detoxified the aflatoxin in the gastrointestinal area. | Khan and Zahoor, 2014 |
| Aflatoxin B1 | 0.3 mg/kg | Sodium selenite | 0.4 mg/kg | Sodium selenite has inhibited AFB1-induced cell apoptosis by reducing Bax and Caspase-3 mRNA expression and increasing Bcl-2 mRNA expression in jejunal mucosa. | Peng et al., 2014 |
| Ochratoxin A (OTA) | 2 mg/kg | Modified zeolite, Esterified glucomannans or mixed of the absorbents | 0.2% of each adsorbent | Adsorbents have partly prevented the negative effect OTA on broiler performance. Accordingly, they have increased BW and prevented FI from reduction. On the other hand, they have not totally reduced the histopathologic changes caused by OTA. | Nedeljković-Trailović et al., 2015 |
| Aflatoxin (AF) | 20 ppb | Nanocomposite (Magnetic graphene oxide with chitosan) | 0.5% | In the chickens fed by AF, the adsorbent has improved the performance, FCR and the organ lesions. And also, the adsorbent has absorbed more than half of the AF in the gastrointestinal area. | Saminathan et al., 2018 |

BW= body weight, FI= feed intake, FCR= feed conversion ratio.

Adsorbents are substances that prevent the absorption of some chemicals through intestines by binding them physically (Nešić et al., 20119). Physical structure of the absorbent such as molecular features, ion distribution, solubility, size, pore dimension and physical structure are important criteria that affect absorption process. Therefore, the effectiveness of adsorbents differs depending on the chemical structures of the agent and the toxin. For example, it has been reported that HSCAS is a strong absorbent against aflotoxicosis while it has the limited effects against zearalenone and ochratoxin. Similarly, although aluminosilicates have higher specificity than activated charcoal and many other adsorbents, their reactions with vitamins and minerals are indicated as a disadvantage. Therefore, it has been stated that the effectiveness of absorption process must be searched in detail according to the properties of the absorbent (Huwing et al., 2001).

It is known that most microorganisms have the capacity for reducing mycotoxins to non-toxic metabolites and for detoxifying them; however, since some microorganisms can exhibit this activity only under certain conditions, practical results may be different. Respectively, it has been proven that the enzyme (epoxidase) produced by a gram-positive anaerobic bacterium isolated from rumen liquid enables deoxynivalenol detoxification. This

enzyme was found to be suitable for use in feed but it has been stated that there must be strict anaerobic conditions and 24 hours passing under these conditions for the reaction to take place. Therefore, this may be related to the fact that no enzyme activation was observed in the researches and an action against deoxynivalenol were not exhibited on different types of animals (Laurain, 2018).

**Table 3.** Mycotoxin adsorbing agents used in ruminants and monogastric animals

| Mycotoxin | Mycotoxin levels | Product | Product inclusion | Animal | Effect of used product | Literature |
|---|---|---|---|---|---|---|
| Aflatoxin B1 (AFB1) | 0.021 mg/kg | AflaDetox | 1% | Dairy cow | In this research there were no differences milk production, compositin and feed intake. To be added 1% AflaDetox in the diet reduced Aflotoxin M1 (AFM1) | Denli et al., 2003 |
| AFB1 | 75 µg/kg | Clay | 1% | Dairy cow | It has removed the negative effects of AFB1 on milk quality (milk yield and DM content of milk) and reduced AFM1 concentration in milk. | Queiroz et al., 2012 |
| AFB1 | 1 µg/mg | Activated charcoal, Montmorillonite or *Saccharomyces cerevisiae* | 0.5% of each adsorbent | Dairy cow | Montmorillonite and *S. cerevisiae* have significantly reduced the adverse effects of AFB1 on gas production in rumen. On the other hand, there were no differences between the groups in terms of in vitro DMD, OMD, VFA and NH3-N content. | Yeanpet et al., 2018 |
| Deoxinivalenol (DON), Diacetoxyscirpenol (DAS), Fuminosins (FM) and Zearalenone (ZEA) | 1021 µg/kg, 1791 µg/kg, 2339 µg/kg, 595 µg/kg | A mixture of the clay with yeast enzymes and mineral adsorbents | 30 g | Dairy cow | There were better liver functions and reproduction parameters, the improved quality and production of milk and the reduced somatic cell number on the day of 100 in the group with mycotoxin and binders additives compared to the group with only mycotoxin additives. | Zouagui et al., 2017 |
| DON | 1 mg/kg | Actived carbon (AC) | 2 g/kg | Piglet | In the model formed for the first time based on absorption kinetics to assess the in vivo activity of mycotoxin binders, AC has totally prevented the absorption of DON by way of small intestines. | Devreese et al., 2014 |
| Mixture of DON (DON, 3-acetyl deoxynivalenol and 15-acetyl deoxynivalenol) | 3 mg/kg (2.6, 0.1 and 0.3 mg/kg, respectively) | A mixture of acid-activated bentonite, alinoptilolite, yeast cell walls and organic acids | 1 kg/t | Piglet | On the 14th day of feeding, the toll-like receptor 4 gene expression in the distal small intestine mucosa was down-regulated by the binder, the daily FI and BWG increased further and there were no performance differences between the groups on day 37. | Jin et al., 2017 |

DM= dry matter, DMD= dry matter digestibility, OMD= organic matter digestibility, VFA= volatile oil acid, $NH_3$-N= ammonia-bound nitrogen, FI= feed intake, BWG= body weight gain.

It is believed that the successful results obtained from the in vitro studies on mycotoxin binders should be supported by in vivo experiments. The future work is expected to shed light on issues such as the interaction of these agents with the nutrients, vitamins and minerals in animal food, the effects of them on one and even multiple mycotoxins and their binding status in practice.

**Table 4.** Mycotoxin biotransforming agents used in livestocks

| Mycotoxin | Mycotoxin levels | Product | Product inclusion | Animals | Effect of used product | Literature |
|---|---|---|---|---|---|---|
| *Aspergillus flavus* FNCC 6002 and | - | Lactic acid bacteria (*Lactobacillus plantarum* G7) or LAB with methionine (M) | 1% or 0.8% (M)+1 (LAB) or 1.2% (M)+1 (LAB) | Poultry | All binder additives prevented the decrease of performance and reduced the organ damage in the broilers feeding with Aflatoxin. | Istiqomah et al., 2016 |
| Aflatoxin B1 (AFB1) | 1 or 5 mg/kg | *Lactobacillus reuteri, L. plantarum, L. pentosus, L. rhamnosus and L. paracasei* and *Saccharomyces cerevisiae* | The probiotic preparation used contained (per 1 kg): $4.5 \times 10^{10}$ *Lactobacillus* cells and $4 \times 10^{6}$ for yeast *S. cerevisiae* | Poultry | Feed contaminated with an AFB1 at a rate of 1 mg/kg has not affected the performance whereas the contamination with AFB1 at a rate of 5 mg/kg has reduced the FI and BWG, caused hepatomegaly and enlarged kidney. The addition of probiotic has partly prevented these negative effects of AFB1 and reduced the AFB1 concentration in the liver and the kidney between 50 and 70%, respectively. | Śliżewska et al., 2019 |
| Zearalenone (ZEA) and Deoxinivalenol (DON), | 596.86 µg/kg, and 796 µg/kg | A mycotoxin biodegradation agent (MBA, composed of *Bacillus subtilis* ANSB01G and *Devosia sp*. ANSB714) | 2 g/kg | Gilt | MBA addition to ZEA and DON-contaminated diets has increased the BWG, reduced plasma immunoglobulin concentration, and suppressed apoptosis by reducing Caspase-3 expression and increasing BcI-2 expression in ovaries. | Shi et al., 2018 |
| Ergot | 2564 or 2563 ppb total alkaloids (R + S epimers) | A commercial binder = Biomin® II (BB) (contained diatomaceous earth, kaolin clay, yeast and plant extracts, and enzymes) | 30 g (oral dose) | Lamb | Mycotoxin binder increased the NDFD and prevented the performance from reduction. On the other hand, it increased the toxin discharge, and it was seen that there were more toxins in the feces of the animals fed with the group including alkaloid + mycotoxin binder additives. | Stanford et al., 2018 |

FI= feed intake, BWG= body weight gain, NDFD= neutral detergent fiber digestibility

## 4. Conclusion

Over the world, the contamination of food and feeds with mycotoxins comprises a significant issue. They created a major risk factor for human and animal health. Due to the frequently occurence of mycotoxins and toxicity, these needs to be detoxified with diverse methods. There are used several methods (physical, chemical and biological) for detoxification of mycotoxins. An alternative also approach to reduce to mycotoxin is the use of adsorbing and biotransforming agents. A mycotoxin binders added to the diet should effectively sequester mycotoxins to prevent toxicity in animals and to prevent absorption by gastrointestinal tract. However, this toxin binder have some positive effects aside from have lots of risks. Because of this, the adverse effects of many mycotoxin binders must be prevented and increased their activity.

Mycotoxin adsorbing agents should effectively absorb mycotoxins, reduce mycotoxin availability, reduce animal toxicity and tissue residues, not be destructive effects, have variable positive results and inclusion in diets, resistant to physical, chemical and biological effects of feed manufacturing and not be expensive. Besides, there is not enough published information on the use of mycotoxin biotransforming agents especially in ruminants. For this reason, more research needs to be done about them.

**Conflict of interest**

The authors declare that there is no conflict of interest.

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