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## Mycotoxin absorbents in dairy cattle

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**Abstract.** Ingestion of mycotoxins by animals causes damage to the production system and can still be transferred to animal products, including milk. Due to its carcinogenic and genotoxic potential, the intake of mycotoxins, especially aflatoxins, is relevant to human health. The use of mycotoxin-absorbing agents has gained attention in dairy cattle nutrition. Therefore, it aimed to conduct a literature review on the use of mycotoxin absorbers in the dairy cattle diet. Mycotoxin absorbing agents can be of organic or inorganic origin, the inorganic ones being the most studied. Inorganic and organic agents have been shown to be effective in reducing the transfer of aflatoxin M1 to milk. However, the inclusion of mixed agents (organic and inorganic) is promising as a potential for mycotoxin absorption. In general, organic, inorganic and mixed absorbents showed positive results in improving the antioxidant and inflammatory status in the liver.

**Keywords:** Toxin, Livestock feed, Mycotoxin absorber

### Introduction

Mycotoxins are a group of toxic compounds produced by filamentous fungi in growing forages or stored rations. These when ingested can reduce food consumption, health and the productive and reproductive performance of domestic and human animals (OGUNADE et al. 2016).

Among mycotoxins, aflatoxin B1 (AFB1) is the most problematic in dairy cows, as its

metabolite, aflatoxin M1 (AFM1) can be transferred to milk (DIAZ et al., 2004). Thus, it may impact the health of consumers of milk and its derivatives.

Many approaches have been employed to reduce the risk of ingestion of aflatoxin by cattle and the resulting aflatoxicosis. Ozonation and ammonization can minimize the production and effects of toxins in corn and cottonseed meal (CAST, 2003). However, these approaches are expensive

and time-consuming (KUTZ et al., 2009), so they are not normally used on dairy farms. Recently, a new approach to research is the use of mycotoxin absorbents. It consists of compounds of great molecular weight capable of reducing the bioavailability of mycotoxin in the gastrointestinal tract.

Therefore, it aimed to conduct a literature review on the use of mycotoxin absorbing agents for dairy cattle.

## Contextualization and Analysis

### *Mycotoxins in dairy cattle production*

Mycotoxins are secondary metabolites of low molecular weight and produced by filamentous fungi that promote adverse effects in humans and animals. The most relevant mycotoxin groups found in feed for animal feed are produced by three main fungi genera: *Aspergillus* (aflatoxins (AFs) and ochratoxin A (OTA)), *Penicillium* (OTA) and *Fusarium* species (trichothecenes, fumonisins (FBs) and zearalenone (ZEN)) (Table 1) (ASSIS et al., 2019).

Mycotoxinogenic fungi can develop on plants in the field or during the storage period (YIANNIKOURIS; JOUANY, 2002). In addition, inadequate harvesting practices, drying, handling, packaging and inadequate transport conditions contribute to increasing the risk of mycotoxin production (BHAT et al., 2010).

Recently, Assis et al. (2019), carried out an extensive review of the action of the main mycotoxins on the metabolism and performance of ruminants. Highlighting that the main harms found were changes in the metabolic state of hepatoprotective enzymes, immunosuppression, reduced digestibility of dry matter, production of AGV's, microbial protein synthesis and pathological disorders such as liver and neurological damage. In the productive performance, there is a reduction in food intake, weight gain, feed conversion, milk production, loss of body weight and in severe cases of intoxication, it can evolve until the death of the animal. In relation to reproductive performance, mycotoxins can promote delayed reproduction, non-synchronized ovarian cycles, reduced conception rate, lower percentages of childbirth, abortion and infertility.

Many diseases in humans have been related to mycotoxin intake, mainly due to chronic consumption. The main toxic effects are: carcinogenicity, genotoxicity, hepatotoxicity, nephrotoxicity, estrogenicity, reproductive disorders, immunosuppression and dermal irritation (ANFOSSI et al., 2010).

The mycotoxin with the greatest carcinogenic potential is aflatoxin B1 (AFB1). And up to 6% of AFB1 in the diet can be transferred to milk

as hydroxy-AFB1 and aflatoxin M1 (GALVANO et al., 1996; EFSA, 2004; UPADHAYA et al., 2010). Thus, aflatoxin M1 (AFM1) represents a safety risk in milk and dairy products (IARC, 2002; LIU; WU, 2010). The maximum amount of AFM1 in milk allowed by the United States Food and Drug Administration (FDA, 2000) is 0.5 µg / kg of milk and by the National Health Surveillance Agency of Brazil (ANVISA, 2011) is 0.5 µg / kg of milk. While the maximum concentration of AFM1 in milk allowed by the European Commission is 0.05 µg / kg (EFSA, 2004).

In this context, there is a need to add substances that promote the inhibition and / or inactivation of mycotoxins contained in the animals' diets. Thus, the use of mycotoxin-absorbing additives seems to be an alternative to circumvent the problems arising from the contamination of mycotoxins in the feed of farm animals.

### *Main mycotoxin absorbents*

Mycotoxin absorbing agents are compounds of high molecular weight that bind to the mycotoxins present in contaminated foods, limiting their bioavailability after ingestion. Mycotoxins can bind to absorbent agents through different types of interactions: hydrophobic bonds, hydrogen bonds, electrostatic attraction or repulsion and coordination bonds (DI GREGORIO et al., 2014).

It is important that the mycotoxin complex and absorbent agent (mycotoxin + absorbent) are stable throughout the digestive tract. Thus, its stability at variable pH and the physical-chemical properties of the toxins is one of the crucial parameters to be evaluated to avoid the desorption of the toxin in the gastric tract (AVANTAGGIATO et al., 2005; HUWIG et al., 2001; KABAK et al., 2006). In general, the absorbent agents most used in animal nutrition can be divided into two groups: inorganic and organic compounds.

### *Inorganic absorbents*

#### *Aluminosilicates*

Aluminosilicates are the most abundant group of rock-forming minerals and the basic structure of silicate clay minerals consists of the association of aluminum tetrahedral and octahedral silica sheets, both having hydroxyl and oxygen groups (DI GREGORIO et al., 2014).

Within this group, there are two main subclasses: phyllosilicates and tectosilicates. Phyllosilicates include bentonites, montmorillonites, smectites, kaolinites and illites. They can absorb substances on their surface or within their interlamellar space. On the other hand, silicate ceilings are formed by zeolites, which provide a large specific bonding surface, as well as size, shape and charge selectivity (HUWIG et al., 2001).

**Table 1.** Main mycotoxins found in food for farm animals

Fungi	Micotoxina
<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	Aflatoxins: AFB1, AFB2, AFG1, AFG2
<i>Aspergillus ochraceus</i> , <i>Penicillium verrucosum</i> , <i>Penicillium viridicatum</i>	Ochratoxina A
<i>Fusarium graminearum</i>	Zearalenone
<i>Fusarium verticillioide</i> , <i>Fusarium proliferatum</i>	Fumonisin: FB1, FB2
<i>Fusarium graminearum</i> , <i>Fusarium sporotrichioides</i> , <i>Fusarium poae</i> , <i>Fusarium equiseti</i>	Trichothecenes: DON, 3- or 15-Ac-DON, NIV (type B), T-2, HT-2 (type A)

3-Ac-DON = 3-acetyloxynivalenol; 15-Ac-DON = 15-acetyloxynivalenol; AF = aflatoxins; AFB1 = aflatoxin B1; AFB2 = aflatoxin B2; AFG1 = aflatoxin G1; AFG2 = aflatoxin G2; DON = deoxynivalenol; FB1 = fumonisin B; FB2 = fumonisin B2; HT-2 = HT-2 toxin; NIV = nivalenol; OTA = ochratoxin A; T-2 = T-2 toxin; ZEN = zearalenone.

#### *Hydrated calcium and sodium aluminum silicate (ACSH)*

ACSH has been shown to act as an enterosorbent that binds strongly and selectively to aflatoxins in the animals' gastrointestinal tract, decreasing their bioavailability and toxicity (HARPER et al., 2010; NEEFF et al., 2013; PHILLIPS et al., 2008).

Evidence suggests that aflatoxins can react at various locations in the ACSH particles, especially in the interim region, but also at the edges and basal surfaces (KOLOSOVA and STROKA, 2011). Furthermore, another form of AFB1 sorption by ACSH surfaces may involve the interaction or chelation of AFB1 with cations (especially Ca) or with various metals (DI GREGORIO et al., 2014).

#### *Bentonites (montmorillonites)*

In the case of bentonites, they are characterized by being phyllosilicate clays with layers of crystalline microstructure of variable composition. They are often called smectites because clay is the dominant mineral. In general, the degree of effectiveness of bentonite absorption depends on the amount of montmorillonite and interchangeable cations in its composition (KOLOSOVA and STROKA, 2011).

Montmorillonite is composed of layers of octahedral aluminum and tetrahedral silicon coordinated with oxygen atoms. The large surface area and high cation exchange capacity of the smectite group make them capable of adsorbing organic substances through the penetration of cations and polar molecules. Bentonites have shown great efficacy in the absorption of mycotoxins, specifically aflatoxins (KONG et al., 2014; MAGNOLI et al., 2011) and other mycotoxins (ZEN, OTA and FBs) in several studies (RAMOS et al., 1996a, b; AVANTAGGIATO et al., 2005; MIAZZO et al., 2005; WANG et al., 2012).

#### *Zeolites*

The zeolite tectosilicates consist of a set of tetrahedra of SiO<sub>4</sub> and AlO<sub>4</sub> joined in several regular arrangements through shared oxygen atoms to form a three-dimensional structure similar to a cage. The partial replacement of Si<sup>4+</sup> by Al<sup>3+</sup> results in an excess of negative charge that is compensated by alkaline and alkaline earth cations,

such as sodium, calcium and potassium ions (DAKOVIC et al., 2003; HUWIG et al., 2001).

Studies have shown that natural zeolite-clinoptilolite can adsorb aflatoxins and other mycotoxins, such as fumonisins (DAKOVIC et al., 2010). However, modified zeolites are more effective than natural ones in relation to the absorption of fumonisins (BAGLIERI et al., 2013).

#### *Organic adsorbents*

##### *Yeast cell wall (YCW)*

YCW consists mainly of proteins, lipids and polysaccharides, such as glucans and mannans, being the two main constituents of the last fraction. YCW exhibits a wide variety of mycotoxin absorption loci, as well as different binding mechanisms such as hydrogen bonds, ionic or hydrophobic interactions (RINGOT et al., 2007).

Studies suggest that PCL has a broader spectrum of mycotoxin sorption, such as ZEN, OTA and FBs (FRUHAUF et al., 2012; PFOHL-LESZKOWICZ, et al., 2015; SHETTY and JESPERSEN, 2006), including DON, being the β-glucan fraction of YCW was directly correlated with the binding process (FAUCET-MARQUIS et al., 2014). The *Saccharomyce cerevisiae* mannans have also been shown to be effective in binding DON at different pH values (CAVRET et al., 2010).

##### *Activated carbon (AC)*

AC is an insoluble powder produced by the pyrolysis of several organic compounds, followed by its chemical or physical activation in order to develop a highly porous structure. *In vitro* data suggest potential affinity for several mycotoxins, however, the *in vitro* efficacy of AC in relation to some mycotoxins has not been confirmed *in vivo* (AVANTAGGIATO et al., 2005).

Generally, the adsorption properties of AC depend on the source materials, surface area and pore size distribution (KOLOSOVA and STROKA, 2011). However, AC is nonspecific, therefore, the essential nutrients for the development of animals such as vitamins and minerals (VEKIRU et al., 2007) are also adsorbed.

#### *Mycotoxin absorbers used in dairy cattle*

Systematically, in Table 2, there follows a compilation of experimental data on the effects of the use of mycotoxin absorbing agents in diets for dairy cattle.

**Table 2.** Use of mycotoxin absorbents in dairy cattle.

Mycot	Absorbent	Animal	Treatments	Result	References
AFB1	SolisMos (AA)	Dairy cows	Toxin (20 or 40 µg AFB1 / kg DM) Ex2: 0.25% AA + 20 µg AFB1 / kg DM Ex3: 0.25% AA + 40 µg AFB1 / kg DM	Both ↑ antioxidant status in the liver (superoxide dismutase) Ex2 ↓ AFM1 in milk and ↑ AGV production..	Xiong et al. (2015)
AFB1	Bentonite clay (BC); Fermentation product <i>Saccharomyces cerevisiae</i> (SC)	Dairy cows	Toxin (1,725 mg AFB1 / animal day) T1 = Toxin + 200 g BC / animal day T2 = Toxin + 35 g mix (BC + SC) / animal day	T1 and T2 ↓ AFM1 concentration in milk. T2 prevented ↓ milk production and T1 avoided ↑ OGT.	Jiang et al. (2018)
AFB1	YCW; DY; AL; BY	Dairy cows	Toxin (480 µg AFB1 / animal day) 20 g / day of YCW 20 g / day of DY 20 g / day of AL 20 g / day BY	↓ 78%, 89%, 45% and 50% of milk AFM1 for YCM, AL, DY and BY, respectively.	Gonçalves et al. 2017
AFB1	Solis NovasilPlus MTB-100	Dairy cows	Toxin (112 µg AFB1 / kg DM) Toxin + 0.56% Solis in diet Toxin + 0.56% of NovasilPlus in DM of diet Toxin + 0.56% MTB-100 in DM of diet	Solis and NovasilPlus ↓ the concentration of AFM1 in milk.	Kutz et al. 2009
AFB1	NovasilPlus	Dairy cows	Toxin (100 µg AFB1 / kg MS) Toxin + 0.5% NovasilPlus in DM of diet Toxin + 1% NovasilPlus in DM of diet	NovasilPlus ↓ the concentration of AFB1 in milk, better values at the dose of 1% DM daily.	Maki et al. 2016a
AFB1	LFPS; HFPS; LFSBC	Dairy cows	Toxin (1,725 µg AFB1 / animal day) 20 g LFPS / animal day 20 g HFPS / animal day 20 g LFSBC / animal day	Absorbents ↓ the time required to ↓ the concentration of AFM1 in milk after withdrawing AFM1 from the diet. Only LFPS prevented the adverse effects of AFM1 on milk and on the production of fat-corrected milk.	Ogunade et al. 2016
AFB1	Calibrin clay A (CAA; montmorillonite)	Dairy cows	Toxin (75 µg AFB1 / kg diet) Toxin + 0.2% CAA in DM of diet Toxin + 1% CAA in DM of diet	Does not hear the effect of the absorbent	Queiroz et al. 2012
AFB1	Argila (vermiculita, não tronita e montmorilonita).	Dairy cows	Toxin (100 µg AFB1 / kg DM) Toxin + 0.5% clay in DM Toxin + 1% clay in DM Toxin + 2% clay in DM	Ruminal clay supplementation reduced the transfer of AFM1 from the rumen to milk and feces.	Sulzberger, et al. 2017
AFB1	Biorigin (yeast and bentonite)	Dairy cows	Toxin (100 µg AFB1 / kg DM) Toxin + 30 g of Biorigin / animal day Toxin + 60 g of Biorigin / animal day	Additives can be beneficial in reducing inflammation during the AFB1 challenge.	Weatherly et al. 2018

↑ = increased; ↓ = reduced; AFB1 = aflatoxin B1; AFM1 = aflatoxin M1; AL = altolized yeast; BY = partially dehydrated brewery yeast; DM = dry matter; DY = dry yeast; HFPS = high-dose fermentation product of *S. cerevisiae*; LFPS = low-dose fermentation product of *S. cerevisiae*; LFSBC = fermentation product of *S. cerevisiae* containing low dose combined with sodium bentonite clay; MTB-100 = yeast-derived cell wall glucomannan; Mycot = mycotoxin; NovasilPlus = calcium montmorillonite clay; OGT = oxalacetic glutamic transaminase; Solis = mixture of aluminosilicate mineral clays; YCW = yeast cell wall.

**Table 2.** Use of mycotoxin absorbents in dairy cattle (continuation)

Mycot	Absorbent	Animal	Treatments	Result	References
AFB1	YCAL MTB-04 MTB-06 MTB-04-06 Astra-Ben 20	Dairy cows	Toxin (94 ppb / DM) Toxin + 100 g YCAL / cow day Toxin + 10 g MTB-04 / animal day Toxin + 10 g MTB-06 / animal day Toxin + 10 g MTB-04-06 / animal day Toxin + 227 g Astra-Ben 20 / animal day	Only Astra-Ben 20 ↓ the concentrations of AFM1 in milk.	Kissell, et al. 2012
AFB1	Sodium bentonite; Activated charcoal	Dairy goats	Toxin (100 ppb of AFB1 / day) Toxin + 1% sodium bentonite from DMI Toxin + 1% activated carbon from DMI	Both ↓ the concentration of AFM1 in the milk.	Rao e Chopra, 2001
AFB1	NovasilPlus	Dairy cows	Toxin (121 ppb of AFB1 / animal day) Toxin + 6 g of NovasilPlus / animal day Toxin + 12.1 g NovasilPlus / animal day	The dose of 6 g ↓ 55% of AFM1, while 12.1 g ↓ 68% of AFM1 in milk.	Maki et al. 2016b
AFB1	Toxy-Nil ou UnikePlus	Dairy cows	Toxin (2.8 mg AFB1 / animal day) Toxin + 100 g of Toxy-Nil / animal day Toxin + 100 g of UnikePlus / animal day	Unike Plus ↓ 52% AFM1 and Toxy-Nil ↓ 63% AFM1 in milk.	Rodrigues et al. 2019
AFB1	FloMatrix (aluminosilicate clay)	Dairy cows	Toxin (100 µg AFB1 / kg MS) Toxin + 113 g FloMatrix / animal day Toxin + 227 g of FloMatrix / animal day	The adsorbents had a positive effect on milk production and inflammation of hepatocytes and ↓ the transfer of AF in milk.	Pate et al. 2018
AFB1	SolisMos	Dairy cows	Toxin (20 µg AFB1 / kg DM) Toxin + 0.25% SolisMos in DM of diet	The additive ↓ the transfer of AFM1 in milk and oxidative stress. Improved immunological condition and rumen fermentation.	Xiong et al. 2018
DON e FB	Mycofix (composed of minerals and enzymes)	Dairy cows	Toxin (897.3 µg DON / kg DM and 1,247.1 µg FB / kg DM) Toxin + 35 g of Mycofix / animal day	^ Digestibility of DM and NDF. ↓ activity of liver transaminases due to high doses of mycotoxins.	Gallo et al. 2020
AFB1	Smectite clay (SA)	Dairy cows	Toxin (2.13 g of FB1 / kg DM in diet) Toxin + 100 g SA / animal day	AE ↓ the concentration of AFM1 in milk at 64.8%.	Gallo et al. 2019
AF	Calcium montmorillonite (CM)	Dairy cows	Toxin (50 ppb of AF) Toxin + 0.125% CM of DMI Toxin + 0.25% CM of DMI	Inclusion of CM ↓ the concentration of AFM1 in milk.	Maki et al. 2017
AFB1	YCAL	Dairy sheep	Toxin (60 µg AFB1 / kg of food) Toxin + 2 g of YCAL / kg of food	↓ absorption of AFB1 and ↑ the elimination of AFB1 and AFM1 in the sheep's feces. There was no effect on the transfer of milk in AFM1.	Firmin et al. 2011

↑ = increased; ↓ = reduced; AF = aflatoxin; AFB1 = aflatoxin B1; AFM1 = aflatoxin M1; Astra-Ben20 = yeast extract, aluminum silicate and sodium bentonite; DMI = dry matter consumption; DON = deoxynivalenol; FB = fumonisins; NDF = neutral detergent fiber; DM = dry matter; MTB-04 = 2004 yeast derived cell wall glucomannan; MTB-06 = 2006 yeast derived cell wall glucomannan; MTB-04-06 = mixture of cell wall glucomannan derived from yeast from 2004-2006; Mycot = mycotoxin; NovasilPlus = montmorillonite calcium clay; YCAL = yeast cell wall extract and aluminosilicate; Solis = mixture of aluminosilicate mineral clays; Toxy-Nil = heat treated sepiolite, calcium propionate, calcium citrate and yeast wall purified oligosaccharides; UnikePlus = heat treated sepiolite, calcium propionate and yeast wall purified oligosaccharides.

### Final considerations

The main mycotoxin absorbing agents can be of organic and inorganic origin. Inorganic are basically clay minerals (aluminosilicates) consisting mainly of silica and aluminum that can be divided into two subclasses: phyllosilicates and tectosilicates. In the group of organic absorbents

there is activated carbon and derivatives of yeast cell wall, where the latter is the most used.

Absorbents based on aluminosilicate clays are more efficient in reducing the transfer of AFM1 to milk. Inorganic absorbents are found mainly in patented formulas such as NovasilPlus products, SolisMos and others.

However, there are few studies with the use of organic absorbents in dairy cattle. Absorbent agents are mainly the basis of yeast cell wall. Apparently, the results have been shown to be effective in reducing the transfer of AFM1 in milk, despite the few studies carried out.

However, studies conducted with the union of organic and inorganic absorbing agents have shown promise. As is the case with the products Toxy-Nil, Unike Plus and Astra-Bem 20 that were efficient in reducing the concentration of AFM1 in milk.

In general, in some studies, organic, inorganic and mixed (organic + inorganic) absorbents. They have shown positive results in improving the antioxidant and inflammatory status in the liver.

Therefore, there are absorbents effective in reducing AFM1 in milk. It is enough to establish whether the formulation of mixtures of aluminosilicate clays with yeast cell wall veins are more efficient as absorbents than solely inorganic or organic. Additionally, additives composed of minerals and enzymes such as Mycofix deserve attention, since it promoted an increase in the digestibility of DM and NDF, it also reduced the activity of liver transaminases due to the high doses of mycotoxins.

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