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Know-edge Nutrition Interview Series

Mycotoxins

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What are mycotoxins?

Mycotoxins are secondary metabolites, of low molecular weight, non-antigenic, complex in structure, ubiquitous in occurrence, heat stable, lipophilic, produced by a wide range of filamentous fungi, infesting agriculture crops before harvest and food and feed ingredients during storage and are toxic to man and livestock.

Fungi are major plant and insect pathogens. Frank growth of fungi on animal hosts produces the diseases collectively called mycoses (eg Aspergillosis), while dietary, respiratory, dermal, and other exposures to toxic fungal metabolites produce the diseases collectively called mycotoxicoses (Bennet and Kilch, 2003).

The major contributors for production of mycotoxins are Aspergillus, Penicillium and Fusarium genera. More than 300 secondary metabolites have been identified although only around 30 have true toxic properties of some concern (Sci Rep to EFSA, 2009). The most common mycotoxins that are of concern are Aflatoxin B1, Fumonisins (especially Fumonisin B1, FB1), Deoxynivalenol (DON), Ochratoxins A, Zearalenone, T-2 and HT-2 toxins. Contamination with multiple toxins is common. In such cases, the effects may be additive and rarely antagonistic. One mold species may produce many different mycotoxins, and several species may produce the same mycotoxin.

Mycotoxins are not accumulated in muscles. The mycotoxin metabolites from the animals are excreted in feces and urine and also in food products i.e in eggs and milk. Regarding the occurrence of mycotoxin residues in eggs, very low AFB1 residue levels (around 0.3 µg/kg) can be found following a contaminated diet at a level as high as 10 000 µg AFB1/kg (10 mg/kg). This is also the case for other mycotoxins such as OTA, T-2, DON, ZEA and FB1 for which no significant carry-over (rate in a range of 0.6-0.001 %) in eggs has been observed. only the occurrence of AFM1 in milk (the “milk aflatoxin” from AFB1 metabolism) is a matter of concern with regard to the transfer of mycotoxins in the dairy food chain. The mean rate of carry-over in milk varies according to the mycotoxins: from 0.3-2.2% for AFB1 (Sci Rep to EFSA, 2009).

About 25% of crops in the world are contaminated with mycotoxins (FAO: Jelinek et al., 1989). Mycotoxins are ubiquitous and accessible in different materials (soils, agricultural crops, feed ingredients, feeds and foods). Under practical conditions, no animal feed is completely free of mycotoxins and no feed can be expected to contain only one type of mycotoxin.

How are they produced?

Mycotoxins may occur in cereal crops, leguminous plants, animal feeds and animal products.

The toxicogenic field fungi may be a. plant pathogens (eg. Fusarium graminearum; mycotoxin: deoxynivalenol, nivalenol); b. grow on stressed or senescent plants (eg. Fusarium moniliforme, mycotoxin: fumonisin; Aspergillus flavus, mycotoxin: aflatoxin); and c. initially colonize the plant before harvest and predispose the products to mycotoxin contamination after harvest (eg. Penicillium verrucosum, mycotoxin: ochratoxin, A. flavus mycotoxin: aflatoxin) (Ayalew, 2010).

Most mycotoxin problems are from the fields (before harvesting). Poor hygienic conditions, high temperature and moisture content during harvesting, processing, transport and storage of agricultural products favor mycotoxin production. Stress to the plants (drought and insect damage) increase the susceptibility of the plants for fungal invasion (See Tola and Kebede, 2016). It is suggested that the agricultural produce including feed ingredients and foods, may be stored at a moisture content not higher than 13% (water activity 0.88 to 0.90) and at low temperatures to prevent infestation from storage fungi (Water activity = Vapor pressure of liquid/Vapor pressure of pure water at same temperature).

Which crops are affected by mycotoxins?

Several crops and their products may contain mycotoxins. Cereals and cereal byproducts (maize, rye, sorghum, barley, oats, wheat, rice, millets, wheat bran, and rice bran), nuts (particularly groundnuts), tree-nuts, cottonseed, coffee and cocoa beans, pasture grasses, grapes and other agricultural crops may contain mycotoxins i.e. secondary metabolites of filamentous fungi. Any food or feed item may contain mycotoxins.

In what way do mycotoxins affect the animals?

Mycotoxins may affect several organs and organ functions. They may be hepatotoxic, nephrotoxic, immunotoxic, oestrogenic, neurotoxic, teratogenic, genotoxic, mutagenic and carcinogenic etc. The mycotoxicosis may manifest in an acute or chronic manner. The chronic manifestation of mycotoxicosis is more common. Mycotoxins influence several organs affecting metabolism, lower immunity, increase mortality and decrease the performance of poultry and livestock (feed intake, growth rate, feed conversion, product yield and quality, reproduction). The toxins may be transferred from feed ingested by the poultry and livestock to the food products they produce. Milk is the important food product of concern for aflatoxin M1.

Which organs of the animal are affected the most?

Several organs (liver, kidney, intestines, thymus, bursa, spleen etc) are affected by different mycotoxins.

By what method can they be destroyed or incapacitated?

Mycotoxins production can be prevented or restricted by good managemental practices in agricultural practices (difficult

to practice practically) and food and feed storage.

The moisture content of the foods, feed and feed ingredients has to be not higher than 12%. They have to be stored at a temperature lower than 25oC. Several antimycotics (eg. propionic acid, propionate, sorbic acid, sorbate, 0.15%, benzoic acid, benzoate, parabens and some herbs (cloves, cinnamon oil, mustard, allspice, garlic, oregano), can be applied to the ingredients in storage. The feeds and feed ingredients can be subjected to fluorescent or ultraviolet (UV) rays and gamma or electronic irradiation to prevent mycotoxin production.

Mycotoxin affected grains or parts can be separated by sorting out the contaminated portion. Formaldehyde-ammoniation process can be used to reduce aflatoxins content in animal feed (Afssa, 2007). Mycotoxin detoxifying agents (mycotoxin binders and mycotoxin modifiers) may be added to poultry and livestock feeds to bind the toxins and to reduce the effects of mycotoxins.

What are toxin binders? How do toxin binders remove toxins?

The mycotoxin detoxifying agents can be divided into two different classes, namely mycotoxin binders and mycotoxin modifiers. These two classes have different modes of action; mycotoxin binders adsorb the toxin in the gut, resulting in the excretion of complex toxin-binder in the faeces, whereas mycotoxin modifiers transform the toxin into non-toxic metabolites (Sci Rep to EFSA, 2009). The extensive use of these additives led, in 2009 in the European Union, to the establishment of a new group of feed additives called mycotoxin detoxifiers. These compounds are specified as “substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action” (Sci Rep to EFSA, 2009).

Mycotoxin-adsorbing agents are large molecular weight compounds that should be able to bind the mycotoxins in contaminated feed without dissociating in the gastrointestinal tract of the animal. The toxin-adsorbing agent complex is eliminated via the faeces. This prevents or minimizes exposure of animals to the mycotoxins.

Mycotoxin-adsorbing agents can be silica-based inorganic compounds (natural clay products as well as synthetic polymers) or carbon-based organic polymers (activated charcoal or carbon products).

Do clays have the capacity to remove toxins? What are the advantages of using bentonite over other clays for the removal of toxins?

Silicate minerals are the largest class of mycotoxin sequestering agents and most studies on the alleviation of mycotoxicosis by the use of adsorbing agents have focused on aluminosilicates (the phyllosilicate subclass (bentonites, montmorillonites, smectites, kaolinites, illites) and the tectosilicate subclass (zeolites).

Bentonites are adsorbing agents with a layered crystalline microstructure and variable composition. Bentonites are generally of impure clay consisting mostly of montmorillonite. Due to their montmorillonite content, bentonites swell and form thixotropic gels (Diaz and Smith, 2005, See Sci Rep to EFSA, 2009).

Montmorillonite is a layered silicate which adsorbs organic substances either on its external surfaces or within its interlaminar spaces (Ramos et al., 1996, See Sci Rep to EFSA, 2009).

Zeolites are crystalline hydrated aluminosilicates of alkali and alkaline-earth cations characterized by an infinite three-dimensional structure. Zeolites are a group of silicates consisting of interlocking tetrahedrons of SiO4 and AlO4- (Kabak et al., 2006; Ramos and Hernandez, 1997; See Sci Rep to EFSA, 2009). Zeolites have large pores that provide space for large cations such as sodium, potassium, calcium… . They are characterized by their ability to lose and absorb water and exchange constituent cations without damage to the crystalline structure (Diaz and Smith, 2005; Papaioannou et al., 2002; See Sci Rep to EFSA 2009). Clinoptilolite is a natural zeolite whose main application is the adsorption of heavy metals from aqueous solutions (Kleiner et al., 2001; See Sci Rep to EFSA, 2009)).

Hydrated sodium calcium aluminosilicate (HSCAS) is a naturally occurring and heat-processed calcium montmorillonite that is commonly used as an anticaking additive in animal feed.

How effective is activated carbon in the removal of toxins?

Activated charcoal (AC) can be produced from a great variety of raw, carbonaceous materials, such as bituminous and sub-bituminous coal, lignite coal, bamboo, coconut husks, and wood and other cellulosic materials. AC completely prevented the absorption of deoxynivalenol (DON) from the intestinal tract of pigs (Devreese et al., 2014).

AC is a non-soluble powder formed by pyrolysis of several organic compounds and manufactured by activation processes aimed at developing a highly porous structure (Galvano et al., 2001). AC is known as one of the most effective and non-toxic group of sorbents and has been shown to be a tenacious adsorbing agent of a wide variety of drugs and toxic agents. It has been commonly used as a medical treatment for severe intoxications since the 19th century (Huwig et al., 2001). The sequestrant properties of AC depend on many factors including pore size, surface area, structure of the mycotoxin and dose. Super-activated charcoal differs from AC in that the particle size is reduced, thereby increasing surface area. The specific surface area of AC indeed varies from 500 m^2/g to 3500 m^2/g for super-activated charcoals (Ramos et al., 1996) (See Sci Rep to EFSA, 2009).

What is the property of activated carbon and clay that is responsible for the removal of toxins?

Activated carbon (AC) is carbonaceous

(composed of carbon atoms), complex in structure, highly porous adsorptive medium. The network of pores in activated carbons are channels created within a rigid skeleton of disordered layers of carbon atoms, linked together by chemical bonds, stacked unevenly, creating a highly porous structure of nooks, crannies, cracks and crevices between the carbon Layers.

AC is manufactured using chemical activation and high temperature steam activation mechanisms from coconut shell, crystal peat, hard and soft wood, lignite coal, bituminous coal, olive pits, pines and various other carbonaceous materials. It is a highly adsorbing material. It is used in many industries and applications e.g. to clean industrial waste water, in medicine, discolouring agent for sugar, animal feed industry and so on. AC has high porous structure to give high surface area (1 kg of AC may have approximately 4000 sq meters of surface area).

AC has millions of tiny pores that can capture, bind, inactivate, and remove up to 100 times the AC's weight in toxins in excess of 3,000 m2 i.e. 32000 ft2 (Dillon et al., 1989), which allows it to adsorb large amounts of chemicals, toxins or poisons (Admin, Mold safe solutions, 2016).

Do organic acids have any role to play in the removal of toxins?

Organic acids (eg. propionic acid) and their sodium salts are antimycotic. They also act as mycotoxin detoxifying agents. Citric acid (Mendez-Albores et al., 2007) and lactic acid have detoxification effect against aflatoxins. A 5% solution of lactic and citric acid reduces the concentration of common trichothecene mycotoxins (Humer et al., 2016).

Does the source of activated carbon have any effect on adsorption of toxins?

The adsorption properties of activated charcoal (AC) are strictly dependent on the source materials and physicochemical parameters, such as surface area and pore size distribution. In vitro it has high ability of binding with several mycotoxins. The adsorption property of AC was found effective against aflatoxin B1 and ochratoxin A up to 95% and 91%, respectively, during in vitro studies (Galvano et al., 2001). The information on the amount of AC to be added to the feed and possibly long-term effects on adsorption of essential nutrients are scanty. AC at 2% level had shown beneficial effects, during in vivo studies. AC derived from seed shells of Jatropha curcas is a potent decontaminating agent for zeralenone (Kalagatur et al., 2017).

What is the method to estimate toxin adsorption capability of activated carbon and clays?

The efficacy of toxin binders or toxin modifiers can be estimated in vitro, using porcine or chicken gastro-intestinal simulation models (39°C for 4h in two buffers at pH 2 and 7) (Avantaggiato et al., 2007; Doll et al., 2007; Gallo and Masoero, 2010; Devreese et al., 2013. Incubation in ruminal fluid may be a preliminary step in ruminant model (at 39°C for 2h at pH 7.0). The concentration of the

mycotoxin or its metabolite can be estimated in plasma (Devreese et al., 2013). Intestinal porcine epithelial cells derived from the jejunum (IPEC-J2) cultivated on TranswellR cell culture inserts were also used to test the ability of mycotoxin binders to bind DON (Devreese et al., 2013).

Does the inclusion of activated carbon in a product allow toxins other than mycotoxins to be adsorbed?

AC (activated carbon, activated charcoal) effectively adsorbs pesticides, environmental hydrocarbons, pharmaceutical agents, mycotoxins, phytotoxins, feed additives, antibacterial and most bacterial toxins (Buck and Bratich, 1986). AC was used successfully to treat a variety of toxicity problems in ruminants (Buck and Bratich, 1986; Bisson et al., 2001; Banner et al., 2000; Poage et al., 2000) and bacterial toxins problems also (Buck and Bratich, 1986).

AC is useful in the removal of E. coli O157:H7 organism and toxin, both in vitro and in vivo (Naka et al., 2001; Marks et al., 1998; Pegues et al., 1979). Naka et al. (2001) reported significant reductions in E. coli O157:H7 concentrations (from 5.33 × 106 to 0.80 × 103 within 5 min) on feeding 5 mg/ml of AC. Initial concentration was reduced below detectable levels with 10 mg/ml.